S. Hrg. 117–555

REVISITING GAIN OF FUNCTION RESEARCH: WHAT THE PANDEMIC TAUGHT US AND WHERE DO WE GO FROM HERE

HEARING

BEFORE THE

SUBCOMMITTEE ON
EMERGING THREATS AND SPENDING
OVERSIGHT

OF THE

COMMITTEE ON
HOMELAND SECURITY AND
GOVERNMENTAL AFFAIRS
UNITED STATES SENATE

ONE HUNDRED SEVENTEENTH CONGRESS

SECOND SESSION

AUGUST 3, 2022

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Committee on Homeland Security and Governmental Affairs

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REVISITING GAIN OF FUNCTION RESEARCH: WHAT THE PANDEMIC TAUGHT US AND WHERE DO WE GO FROM HERE

WEDNESDAY, AUGUST 3, 2022

U.S. SENATE,
SUBCOMMITTEE ON EMERGING THREATS AND
SPENDING OVERSIGHT,
OF THE COMMITTEE ON HOMELAND SECURITY
AND GOVERNMENTAL AFFAIRS,
Washington, DC.

The Subcommittee met, pursuant to notice, at 2:30 p.m., via Webex and in room 342, Dirksen Senate Office Building, Hon. Rand Paul, presiding.

Present: Senators Ossoff, Paul, Scott, Hawley, and Johnson.

Also present: Senator Marshall.

OPENING STATEMENT OF SENATOR PAUL[1]

Senator PAUL. I call this meeting of the Senate Homeland Security and Governmental Affairs Subcommittee on Emerging Threats and Spending Oversight (ETSO) to order. I want to thank Senator Hassan for allowing this hearing to occur.

Welcome to each of our panelists. Thank you for joining us.

The purpose of this hearing by the Subcommittee on Emerging Threats and Spending Oversight is to discuss, as our name implies, the emerging threat posed by gain-of-function research. We will hear from a panel of three witnesses, all of whom are extraordinarily accomplished experts in the scientific community. We are grateful for their work and we are grateful to each of you for taking the time to appear with us this afternoon.

Gain-of-function (GOF) research is a controversial scientific research method involving the manipulation of pathogens to give them a new aspect or ability, such as making viruses more transmissible or dangerous to humans. Despite all we have learned about the potential risks of this particular method of research, this is the first congressional hearing on this subject since the pandemic began.

Today we will discuss what gain-of-function research entails, how gain-of-function research is defined, and whether the definition of gain-of-function research is applied consistently by the Department of Health and Human Services (HHS) Potential Pandemic Pathogens (P3CO) Review Committee. This is a committee that was set up to study potential pandemic pathogens.

[1] The prepared statement of Senator Paul appears in the Appendix on page 35.

We will discuss the responsibility for how we determine the risks and benefits. We will also discuss how this committee operates, how this committee approves or denies projects from receiving Federal funding based on whether the pathogen is considered to be a credible source of potential future human pandemic, and if the potential risks, as compared to the potential benefits to society, are justified. In other words, a project is not gain-of-function if the review committee is unsure if a recombinant virus will create a future pandemic.

There is a question of whether or not there is a reasonable expectation that it might be or whether or not it has been in the past, or what viruses should be and should not be experimented upon. This broad criterion gives one sole committee, which is comprised of an unknown group of bureaucrats—I believe the names are not released of who is on the committee so there is not necessarily any oversight of the oversight—the power to spend millions of taxpayer dollars on a single, preemptive guess, with potentially devastating consequences.

Today we will also consider whether gain-of-function research was performed at the Wuhan Institute of Virology (WIV). First, no one, not myself or anyone I am aware of, argues that a recombinant super-virus that has been published in scientific journals is Coronavirus Disease 2019 (COVID–19) or a close relative. If—and I underline "if"—COVID–19 leaked from the Wuhan lab, it would be a laboratory-created virus that the Wuhan scientists have not yet, and are unlikely ever to reveal.

I maintain that the techniques that the National Institutes of Health (NIH) funded in Wuhan to create enhanced pathogens may have or could have been used to create COVID–19. The American people deserve to know how this pandemic started and to know if the NIH funded research that may have caused this pandemic.

Gain-of-function research has the potential to unleash a global pandemic that threatens the lives of millions. Yet this is the first time the issue has been discussed in a congressional committee.

I am sure each Member of this Committee, as well as the full Senate, can agree that we need stronger government oversight of how our tax dollars are being used to finance experimenting with possibly fatal diseases.

Again, I thank each of our distinguished witnesses for being here today and I thank Chair Hassan for working with me to convene this meeting.

Before we begin I would like to note that I have invited Senators who are not on the Subcommittee to also attend today. Therefore, I ask unanimous consent (UC) to allow Senator Marshall and Senator Johnson to fully participate in the hearing, provided that any Members of the Subcommittee be given deference in the order of recognition. Without objection.

Next I would like to remind witnesses that any written testimony they have, anything that they have submitted, will be included in the record, and to please keep your opening remarks to around 7 minutes.

With that I am going to introduce the witnesses, and we will hear their remarks after the introduction, which is slightly dif-

ferent than we do sometimes, and then I will introduce the next witnesses.

The first witness will be with via WebEx. It is Dr. Richard Ebright. Dr. Richard Ebright is the Board of Governors Professor of Chemistry and Chemical Biology and the Director of the Waksman Institute of Microbiology at Rutgers University. Dr. Ebright completed his undergraduate degree from Harvard University in biology, where he earned summa cum laude honors. He later received a PhD in microbiology and molecular genetics, also from Harvard.

Dr. Ebright's research has led to over 175 publications as well as over 40 issued and pending patents. He has received numerous awards for research and is currently a member of the American Academy of Arts and Sciences as well as the Institutional Biosafety Committee at Rutgers University. He is a Fellow of the Infectious Disease Society of America, the American Academy of Microbiology, American Association for Advancement of Science. He was the editor of Molecular Biology for 16 years.

Dr. Ebright currently serves as the project leader of three current NIH research grants, has provided testimony to the House Committee on Energy and Commerce on the 2014 anthrax incident, and was a founding member of the Cambridge Working Group, whose cautionary statement on gain-of-function research involving potential pandemic pathogens remains as relevant as the day it was released in July 2014.

Dr. Ebright.

TESTIMONY OF RICHARD H. EBRIGHT, PH.D.,[1] LABORATORY DIRECTOR, WAKSMAN INSTITUTE OF MICROBIOLOGY, RUTGERS UNIVERSITY

Mr. EBRIGHT. Thank you. Chair Hassan and Members of the Committee, thank you for inviting me to discuss gain-of-function research and its oversight. I am Board of Governors Professor of Chemistry and Chemical Biology at Rutgers, the State University of New Jersey, and Laboratory Director at the Waksman Institute of Microbiology. In my oral statement I will discuss the definition of gain-of-function research of concern, risks and benefits of the research, U.S. oversight of the research, and steps to strengthen U.S. oversight of the research.

What is gain-of-function research of concern? Gain-of-function research of concern is defined as research activities reasonably anticipated to increase a potential pandemic pathogen's transmissibility, pathogenicity, ability to overcome immune response, or ability to overcome a vaccine or drug.

Gain-of-function research of concern involves the creation of new health threats, health threats that did not exist previously and that might not come to exist by natural means for tens, hundreds, or thousands of years.

Gain-of-function research of concern is a small part of biomedical research. It constitutes less than one-tenth of 1 percent of biomedical research and less than 1 percent of virology. However, because gain-of-function research of concern can cause pandemics,

[1] The prepared statement of Dr. Ebright appears in the Appendix on page 38.

this small part is highly consequential and requires effective oversight.

What are the risks? Gain-of-function research of concern poses high, potentially existential, risks. Gain-of-function research of concern poses material risks by creating new potential pandemic pathogens. If a resulting new potential pandemic pathogen is released into humans, either by accident or deliberately, this can cause a pandemic.

Gain-of-function research of concern also poses information risks, by providing information on the construction and properties of new potential pandemic pathogens. Publication of the research provides instructions, step-by-step recipes that can enable a rogue nation, organization, or individual to construct a new pathogen and cause a pandemic.

What are the benefits? Gain-of-function research of concern provides limited benefits. Gain-of-function research of concern can advance scientific understanding, but gain-of-function research of concern has no civilian practical applications. In particular, gain-of-function research of concern is not needed for, and does not contribute to, the development of vaccines and drugs. Companies develop vaccines and drugs against pathogens that exist and circulate in humans, not against pathogens that do not yet exist and do not circulate in humans.

What should oversight entail? Because gain-of-function research of concern poses high, potentially existential risks and provides limited benefits, the risk-benefit ratio for the research almost always is unfavorable and in many cases is extremely unfavorable. Therefore, it is imperative that gain-of-function research of concern be subject to national or international level oversight to ensure before the research is started that risk-benefit ratios are acceptable and risks are mitigated.

Effective oversight includes three components. First, research proposals that include gain-of-function research of concern must be identified and flagged. Second, a risk-benefit assessment must be performed. This entails enumerating risks and benefits, weighing risks and benefits, and reaching a decision, either to proceed as proposed or to proceed with additional risk mitigation, or not to proceed.

Third, compliance with the decision from the risk-benefit assessment must be mandated, monitored, and enforced.

I turn now to U.S. oversight of gain-of-function research of concern.

Before 2014, there was no national-level U.S. oversight of gain-of-function research of concern. In 2014 to 2017, the government put in place a moratorium on Federal funding for "selected gain-of-function research," defined as research activities reasonably anticipated to increase the transmissibility or pathogenicity of influenza, severe acute respiratory syndrome (SARS), or Middle East respiratory syndrome (MERS) viruses. The policy was referred to as the Pause.

Under the Pause, 18 projects were paused. However, at least 7 of the 18 projects that were paused were allowed to resume almost immediately. More important, other projects that met the definition for coverage, including a project on SARS-related coronaviruses by

EcoHealth Alliance and its Wuhan-based partners, were not paused, due to the failure of the NIH to identify and flag all covered projects.

At the end of 2017, the Pause was lifted and was replaced by an HHS policy that requires risk-benefit assessment before awarding HHS funding for "research involving enhanced potential pandemic pathogens," defined as research activities reasonably anticipated to increase the transmissibility or pathogenicity of a potential pandemic pathogen. The policy is referred to as the P3CO Framework.

Under the P3CO Framework, covered projects must be identified and flagged by the funding agency, the NIH, and covered projects must be reviewed by an HHS Secretary-level committee, the P3CO Committee.

The P3CO Framework assesses the reasonably anticipated results of the proposed research. The reasonably anticipated standard employed by the policy is equivalent, in all respects, to the reasonable person standard employed in U.S. administrative law and U.S. civil law.

In principle the P3CO Framework ensures risk-benefit assessment of gain-of-function research of concern. However, in practice, the P3CO Framework has existed primarily on paper. In the 4½ years since the policy was announced, only three projects have been reviewed. Most covered projects, including the project by EcoHealth Alliance and its Wuhan partners, were not reviewed, due to a failure by the NIH to identify and flag covered projects.

In addition, the P3CO Committee has been non-transparent and unaccountable. The names and agency affiliations of its members have not been disclosed, its proceedings have not been disclosed, and even its decisions have not been disclosed.

Current U.S. oversight of gain-of-function research of concern thus has serious shortcomings. Moving forward, any effective system of U.S. oversight of gain-of-function research of concern must address these shortcomings. My recommendations are as follows:

First, responsibility for U.S. oversight of gain-of-function research of concern should be assigned to a single, independent Federal agency that does not perform research and does not fund research.

Second, U.S. oversight of gain-of-function research of concern should cover all U.S. and U.S.-funded research, irrespective of funding source, classification status, and research location.

Third, U.S. oversight of gain-of-function research of concern should be codified in regulations with force of law and should be mandated, monitored, and enforced.

Fourth, U.S. oversight of gain-of-function research of concern should be transparent and accountable.

Thank you for your attention, and I would be pleased to address questions.

Senator PAUL. Thank you, Dr. Ebright.

Next we will have Dr. Steven Quay. Dr. Steven Quay is the Founder and Chairman of the Seattle-Based Atossa Therapeutics. Atossa Therapeutics is a clinical-stage biopharmaceutical company that develops novel therapeutics and delivery methods for breast cancer and other breast conditions, with the goal of preventing the two million yearly breast cancer cases worldwide.

Earlier in his career, Dr. Quay received his MD and PhD from the University of Michigan, trained as a postdoctoral fellow at Massachusetts Institute of Technology (MIT), and served on the faculty of Stanford University's School of Medicine.

Dr. Quay's published contributions to the world of medicine have been cited extensively, and he is a medical entrepreneur. He has founded six startups, invented seven Food and Drug Administration (FDA)-approved pharmaceuticals, and is the holder of 87 patents and over 130 pending U.S. and foreign patent applications.

He is also an author. Notably, during the pandemic, Dr. Quay published his No. 1 Amazon best seller, Stay Safe: A Physician's Guide to Survive Coronavirus.

Finally, Dr. Quay recently presented testimony to lawmakers as part of an expert forum convened by the House Select Committee on Coronavirus, titled "Led by Science: The COVID–19 Origin Story."

Dr. Quay.

TESTIMONY OF STEVEN QUAY, MD, PH.D.,[1] CHIEF EXECUTIVE OFFICER, ATOSSA THERAPEUTICS, INC.

Dr. QUAY. I am honored to participate with my esteemed colleagues, Doctors Ebright and Esvelt, in this forum entitled "Revisiting Gain-of-Function Research: What the Pandemic Taught Us and Where Do We Go From Here."

I offer six statements in opening. One, there is no dispositive evidence the pandemic began as a spillover of a natural virus in a market. All evidence is consistent with a laboratory-acquired infection. I do understand this conclusion is not widely held, I can spend an entire hearing painstakingly going through the scientific evidence for this conclusion, but that is not the purpose of today's meeting.

I am happy to discuss the evidence contained in my written remarks during questioning. I am also willing to publicly debate any virologist on this question, at any time or place. Only one infectious disease doctor was willing to debate this question with me last year in a formal debate format, and he lost.

I am also willing to testify under oath, if requested.

No. 2, all evidence is consistent with an accidental and not a deliberate release.

No. 3, SARS2 has features consistent with synthetic biology gain-of-function research. Two features involve acceptable academic gain-of-function research, the receptor binding domain optimization and the furin cleavage site. These two features have never been found in nature and related viruses that could have reasonably started the pandemic because of the closeness of these viruses to Wuhan.

These two features are, on the other hand, routinely engineered into viruses. In 2018, United States and WIV scientists proposed inserting "human-specific furin cleavage sites in a bat virus backbone." Two years later, SARS2 appeared on the WIV's doorstep. SAR2 is a bat-derived virus with a human-specific furin cleavage site.

[1] The prepared statement of Dr. Quay appears in the Appendix on page 66.

One region of SARS2, called open reading frames (ORF8), has features of forbidden gain-of-function research, asymptomatic transmission and immune system evasion. The WIV was engineering a protein related to ORF8 to have these two forbidden properties before 2019, as shown in two master's degree theses available only in Chinese.

COVID exhibits 40 percent asymptomatic transmission, unheard of for a new respiratory virus. Patients infected with an acquired deletion of ORF8 have a milder infection. Could the reduced efficacy of vaccines and natural immunity be an engineered feature? It appears likely.

Six, in December 2019, the Wuhan Institute of Virology was conducted synthetic biology research on the Nipah virus, which is 60 percent lethal in low-containment, biosafety level 2 (BSL–2), 3 facilities. The Nipah virus was in an infectious clone format. Nipah is a BSL–4 level pathogen and a Centers for Disease Control and Prevention (CDC)-designated bioterrorism agent. This is the most dangerous gain-of-function research I have ever encountered. We should assume this research continues to this day at the WIV.

I will close with five recommendations for future gain-of-function research.

Where did the pandemic begin? The competing hypotheses are a natural spillover at the Hunan Seafood Market in Wuhan and a laboratory-acquired infection. Two recent papers purport to claim the pandemic began at the Hunan Market in December 2019. There are at least six serious problems with these papers.

The most important are that in the early months no animal has ever been found to be infected with COVID–19 anywhere, including the market, and the molecular clock of SARS2 places the first human infection in the fall of 2019, long before the December market cases. All infections in the market in human were what is called Lineage B, and not the most ancestral lineage, Lineage A. I, like many other scientists, believe the market cases were a superspreader event, on this first chart here.

The earliest cluster of hospitalized patients with both the Lineage A and B virus was at the People's Liberation Army (PLA) Hospital in Wuhan. This hospital is about six kilometers from the WIV, and on Line 2 of the Wuhan subway system, as shown in this chart. All early cases are in hospitals adjacent to Line 2, and the probably that this was a chance occurrence is 1 in 68,000.

The Line 2 Covid Conduit, as I called it, includes the PLA Hospital, the WIV, the market, and the international airport. You can literally walk down into the subway system from the WIV in China and next exit outside in London, Paris, Dubai, Los Angeles, or New York, all before having any symptoms. Modeling by others suggests that the pandemic could not have occurred without the international spreading impact of Line 2.

Has gain-of-function research been useful to the COVID response or any other public health infectious disease emergency? I have found no evidence that gain-of-function research helped in either the COVID pandemic or other smaller epidemics.

We now know that an Messenger Ribonucleic acid (mRNA) vaccine can be designed within literally days of a new outbreak once the pathogen is sequenced, and large-scale manufacturing can

begin soon thereafter. This capability has now been fully road-tested and provides, in my opinion, the best defensive capability against future microbes.

It is also important to point out that gain-of-function research is a tiny sliver of all research funded by NIH. Specifically, there were over 36,000 Research Project (RO1) grants funded by NIH in 2020, the latest year with statistics. Of these, the self-described gain-of-function on potential pathogens research grants numbered only 21 in the latest funding year. Even expanding this by tenfold with a less stringent definition of gain-of-function would mean we are talking about less than 1 percent of all NIH research funding. I cannot imagine a scenario where but for this tiny research effort a new pandemic occurs.

What reforms should be considered in order to assure that such research is conducted in a safe and transparent manner? While I found no actual benefit of gain-of-function research, I believe efforts to ban it, given the vested interests of literally the entire virology community, is a hill too steep to climb. A proposal that I believe is achievable is the placement of all select agent research within the existing institutional review board structure used for human clinical trials. I believe this effort would put guardrails around the most dangerous aspects of this research, and has the added benefit of international acceptance, including in China.

My second reform would be to separate government oversight from the funding agency, and the model would be the Atomic Energy Commission.

My third suggestion is to place Western biotechnology equipment under export controls and monitoring. There are ways to build into these systems a forensic and law enforcement capability that could, for example, with probable cause and a court-ordered search warrant allow the work of any lab in the world to be scrutinized remotely.

My fourth recommendation is simple: do not put dangerous infectious disease laboratories near subways, like Line 2, where every major city in the world is accessible with the incubation period of an infection.

Finally, I am including what I call gain-of-opportunity research, going into caves where humans are seldom found, taking a bat fecal sample containing thousands of viruses, bringing those viruses back to a laboratory, and culturing the specimens where a virus might be controlled in a diverse natural environment, is now able to grow unrestricted in pure culture, provides an immense increased potential risk, a gain of opportunity for the virus.

This is the goal of the Global Virome Project, a Gates Foundation-funded, Eco-Health Alliance-associated effort. Their stated goal: collect the estimated 500,000 unknown viruses that are capable of infecting humans and bringing them back to a laboratory near you. What could go wrong?

Could I have the last slide here.

What happens if we have these hearings and nothing happens? In December 2019, we performed a remote audit, forensic examination of the Wuhan Institute of Virology and found synthetic biology experiments with the Nipah virus. As the chart shows, they had created a cloning vector with a virus the U.S. CDC defines as a bio-

terrorism agent. Nipah virus is one of the deadliest on the planet, with a greater than 60 percent lethality.

Why were they conducting this experiment? I do not know. But laboratory-acquired infection with this virus, if it became airborne, would make COVID–19 look like a walk in the park.

The work of this Committee is critical to protecting the American people as well as the people of all countries from future pandemics, manmade or natural. If we now fail to act with the knowledge we have, history will judge us poorly.

Thank you for the opportunity to speak.

Senator PAUL. Thank you, Dr. Quay. Our final witness is Dr. Kevin Esvelt. He is currently an Assistant Professor at the MIT Media Lab group, where he leads the Sculpting Evolution Group.

Dr. Esvelt received his BA in chemistry and biology from Harvey Mudd College and would later complete his PhD in biochemistry at Harvard University, as a Hertz Fellow.

While working in the laboratory of David Liu at Harvard University, Dr. Esvelt invented phage-assisted continuous evolution (PACE), which is a synthetic microbial ecosystem for rapidly evolving biomolecules. Later, during his time as a Wyss Technology Fellow, Esvelt's focus centered around the development of gene drive technology. Many of Esvelt's contributions related to the bioethics and biosafety of such gene drivers, and he is credited as the first to describe how Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene drives could be used to alter the traits of wild populations in an evolutionary stable manner.

In his recent work at the Sculpting Evolution Group, Dr. Esvelt and his colleagues invented the new technology known as "daisy drives," which would let communities aiming to prevent disease alter wild organisms in local ecosystems.

Throughout his career, Dr. Esvelt has been a champion of universal safeguards, transparency, raising scientific awareness of developing early warning systems to reliably detect any catastrophic biological threat, and advising policymakers on how to best mitigate global catastrophic biorisks.

Dr. Esvelt.

TESTIMONY OF KEVIN M. ESVELT, PH.D.,[1] ASSISTANT PROFESSOR OF MEDIA ARTS AND SCIENCES, MIT MEDIA LAB

Mr. ESVELT. Chair Hassan, Ranking Member Paul, Senators, thank you for the kind invitation. I have to say that I have no special insights regarding the origins of COVID. In fact, I kind of doubt that there is sufficient evidence to be conclusive in one way or the other. But our models suggest that knowing where it came from would not actually help us defend against future pandemics.

I agreed to speak to a bipartisan hearing today because this is the Emerging Threats Subcommittee, and I am increasingly concerned by our continuing failure to recognize an increasingly dire technological threat.

Leo Szilard who invented the nuclear chain reaction and launched the modern nonproliferation movement, is a scientific

[1] The prepared statement of Mr. Esvelt appears in the Appendix on page 73.

hero of mine, and he wrote, "The most important step in getting a job done is the recognition of the problem."

The problem is not our inability to agree on what does or does not constitute gain-of-function research or even whether the putative benefits of this research outweigh the risks of accidents. Rather, the problem is that we are so used to thinking of pandemics as a health and safety issue that we have missed the national security implications of identifying viruses that could be deliberately unleashed to kill millions of people.

Let me illustrate. When the genome of SARS2 was first posted online, scientists did not have to wait for physical samples of the virus to become available to begin studying it and working on countermeasures. That is because we could order synthetic deoxyribonucleic acid (DNA) corresponding to the genome of the pathogen and assemble infectious samples using freely available, step-by-step protocols.

From a biomedical perspective, that is a triumph, particularly because it only costs a few thousand dollars, and the price is plummeting. But from a security perspective, that means that thousands of researchers could gain access to a novel pandemic agent, as soon as it was identified as such.

Thankfully, we still do not know of any particularly concerning examples, that is, agents that would likely cause a pandemic if they were to be released, even at multiple sites. If we did know, then the modern-day equivalent of a terrorist, like Seiichi Endo, who is a graduate-trained virologist and doomsday cultist, who sought samples of Ebola and used chemical weapons to commit mass murder, might have well assembled them and released them in airports by now.

But if you work in public health and infectious disease you naturally want to know what the next threat might be so that you can better prepare defenses. That makes sense. That is why both United States Agency for International Development (USAID) and NIH have funded research attempting to find or create novel pandemic-capable viruses in labs all over the world.

Now we disagree on whether some of those experiments might fall into an arbitrarily defined category called gain-of-function research. We biologists disagree over what a species is. Did you know that a tiger and a lion can interbreed? But what nobody disputes is that in the hope of preventing natural pandemics both agencies seek to identify viruses that could kill as many people as a nuclear weapon, to alert the entire world to what they find, and to publicly sharing the complete genome sequences of those viruses so that skilled scientists everywhere will be able to make infectious samples.

The tragedy is that these are health experts, well-meaning health experts, who have dedicated their lives to fighting infectious disease, and they struggle to imagine anyone evil enough to deliberately cause one. They never considered that these advances in technology, which are continuing, plus a list of pandemic-capable viruses, would allow a single skilled terrorist to unleash more pandemics at once than would naturally occur in a century. No one warned them, perhaps because, as has been previously noted, they lack independent security oversight of their work.

Now it is always possible that we could save more lives by helping to prevent natural pandemics than we would lose due to deliberate acts of terrorism. But according to our numerical cost-benefit model, it is not even close, even for the best-case scenario. The reason is there are so many viruses in nature, most of which will never encounter a human. The lowest published estimate suggest that for every pandemic virus that does spill over in a century there are 100 that will never encounter a human.

That means if you identify one at random, even if we could perfectly prevent it from spilling over and causing a pandemic, that one virus, then we have a 1-in-100 chance of actually preventing a pandemic. But if there is just a 1 percent chance of deliberate misuse per year, then in that same time period we can expect to cause a pandemic. In other words, pandemic virus identification, whether it is created in the lab or whether it is just identified in the wild, is expected to kill 100 times as many people as it would save.

For 75 years, the United States successfully kept nuclear weapons out of the hands of terrorists. In the wake of a pandemic that has killed more people than could any thermonuclear explosion, it is time to start doing the same for pandemic viruses.

For starters, Congress could study the issue and release a finding on whether pandemic virus identification endangers national security. It is just that simple. Then, if necessary, reform USAID and NIH research. It could require an oversight committee of experts from security agencies to review all requests for proposals in the life sciences. It could update the Federal Select Agent Program to automatically regulate viruses at the first sign of pandemic capability, because these are the most dangerous agents out there. It could require all DNA synthesis orders to be screened for hazards.

Perhaps most important, Congress could legislate catastrophe liability, that is, liability for human-caused events that result in more than 1 million American casualties, as SARS2 has, and require general liability insurance to cover it. That would induce the market to price in the cost of negative externalities and cause professional insurance risk analysts to perform those cost-benefit analyses.

Now I am optimistic about this issue because we just need to buy time. If we can keep pandemic-capable viruses out of the hands of terrorists for a decade then we can deploy new, general-purpose defensive technologies. These range from ubiquitous sequencing that can detect any emerging threat, to perfect protective equipment for our essential workers, to low-wavelength germicidal lights, and these together could protect us from all pandemics, whether natural, accidental, or deliberate.

Pandemic proliferation is a solvable national security problem, but only if we recognize it as one. Thank you.

Senator PAUL. Thank you, Dr. Esvelt. We will start with Senator Scott from Florida.

OPENING STATEMENT OF SENATOR SCOTT

Senator SCOTT. All right. Thank you, Chair.

Dr. Esvelt, in your testimony you talk about USAID funding gain-of-function experiments through Discovery & Exploration of

Emerging Pathogens—Viral Zoonoses (DEEP VZN), the program which specifically conducts experiments geared toward pandemics and virology and Strategies to Prevent (STOP) SPILLOVER, which as you know, research is spillover between animals and humans. Can you talk about what these programs specifically are and why they may be dangerous?

Mr. ESVELT. DEEP VZN and STOP SPILLOVER are extensions of USAID's long-running PREDICT program, the goal of which was to predict pandemics, that is, to identify viruses in the wild that had a good chance of spilling over and causing a pandemic in humans. This is part of the laudable One Health program which seeks to identify essentially hotspots where viruses are likely to spill over into humans and cause a pandemic. The idea is if we find these hotspots, educate the community, teach them what to do in the event of an outbreak, then we might be able to stop it before it reaches our shores. That makes sense.

But again, they do not seem to have thought of the security issues associated with publishing a list of pandemic-capable viruses, by threat order. Now we cannot necessarily know whether a given pandemic would take off until it is spreading in humans, but there is a narrow set of laboratory experiments that can tell us, does it look like a human endemic virus, in certain traits? These are a tiny subset of all experiments that really are not very useful for anything else. They do not help with therapeutic development.

Part of PREDICT was to take samples of these viruses, bring them back to the lab, run these kinds of experiments, sequence the genomes, share them. They did not find anything particularly scary, but they found some candidates that looked fairly nasty, including at the Wuhan Institute of Virology. It is hard to know what USAID did and did not approve, but they are listed as an acknowledgement, as is NIH, on a paper that recombined those dangerous-looking but definitely not pandemic-capable viruses, and then performed experiments to see, did they look like they could plausibly cause prescription drugs.

Senator SCOTT. Do you think these programs are dangerous?

Mr. ESVELT. I think any program attempting to identify an agent that would be widely accessible and could be deliberately released to kill millions of people is pretty much the definition of dangerous, yes.

Senator SCOTT. Do you think that USAID, whose main job is to provide humanitarian aid globally, has the oversight for programs and experiments like STOP SPILLOVER and DEEP VZN, which are not humanitarian in nature?

Mr. ESVELT. I think there is a very strong humanitarian case for preventing pandemics. I think that the absence of security oversight means that USAID was probably just not aware of the security consequences of their work, and it remains to be seen whether they will decide that it is inadvisable to maintain a ranked-order list of those most threatening viruses.

Senator SCOTT. Do you think they have the oversight ability to handle this job?

Mr. ESVELT. It is unclear exactly who they are seeking advice from. My understanding is that they are seeking advice from folks

with greater security expertise, and the real question is what actions are going to come of that.

Senator SCOTT. Would these programs go through a P3CO review?

Mr. ESVELT. My understanding is that federally funded research does go through P3CO review. However, it is unclear whether the basic find-the-pathogens program, would go through such review because until you find it and at least run some characterization to determine whether or not it looks like a pandemic virus it would not necessary be regulated. As previously mentioned, due to the transparency issues with that committee it is very much unclear what their remit is and is not.

Senator SCOTT. Do you know who is on the panel for P3CO?

Mr. ESVELT. I do not.

Senator SCOTT. Is it not public?

Mr. ESVELT. My understanding is it is not public.

Senator SCOTT. Why would it not be public?

Mr. ESVELT. That is an excellent question.

Senator SCOTT. Do any of the witnesses know why it would not be public?

Mr. ESVELT. No.

Dr. QUAY. No. I know it is not public and I do not know why it is not public.

Senator SCOTT. That is part of our Federal Government, right?

Dr. QUAY. Correct.

Senator SCOTT. Do they think Americans are not smart enough to understand it?

Dr. QUAY. You will have to ask the people at the NIH.

Senator SCOTT. Do you know how they made the decision not to make the names public?

Dr. QUAY. No.

Senator SCOTT. OK. For each of you, do you think that the P3CO review is comprehensive enough on NIH grants or do you think gain-of-function grants have been approved without a P3CO review?

Senator PAUL. Let us go to Dr. Ebright. I do not want to leave him out. Then we will go to each of you. Dr. Ebright, would you like to respond to that?

Mr. EBRIGHT. Yes. As I mentioned in my summary statement, there have been only three P3CO reviews in the 4½ years that the P3CO Framework has been in effect. The majority of gain-of-function research of concern enhanced potential pandemic pathogen research supported by NIH has not undergone P3CO review. It has not undergone P3CO review for the simple reason that the NIH has not identified and flagged the proposals as subject to P3CO review and has not forwarded the proposals for P3CO review.

Senator PAUL. Let me ask the other two to respond as well.

Dr. QUAY. Yes. I think, just echoing Dr. Ebright, it has been a failure, I think, at this this point in time, and so we need to find an alternative, which is perhaps to take it out of the NIH, make the oversight outside of the agency that is funding.

Mr. ESVELT. One major problem is that gain-of-function is a terrible term. It applies to most of biotechnology in the raw. You can try to add qualifiers as you want. But it also inherently does not

catch efforts to identify perfectly natural but nevertheless highly lethal pandemic-capable viruses. It really does not matter where the thing comes from. What matters is do you know that there is a good chance that it causes a pandemic.

Again, maybe you do not think we can ever be confident more than, say, 50 percent for a given virus, but if you get a list of eight viruses that you are 50 percent confident, it is possible to make all eight, let them go, and you have pretty good odds there.

I am concerned by efforts to continue to focus on gain-of-function because it is so ill-defined, and it seems more productive to narrow in on the classes of experiments that can substantially increase our confidence that a virus is pandemic capable, wherever it comes from. I certainly echo the calls for external security oversight.

Senator SCOTT. Do you think there is appropriate oversight of existing research after it has been approved, to ensure continuous compliance?

Mr. EBRIGHT. I would say that there is not. Importantly, the P3CO Framework does not mandate compliance. If the P3CO committee makes a decision that the research may not proceed, that decision is only advisory to the funding agency. It is not mandated for the funding agency. The funding agency is free to accept or not accept the decision, and it is free to determine whether to monitor or not to monitor the progress of the work. This is a major shortcoming.

Senator SCOTT. Thank you.

Senator PAUL. I want to interject on the definition, whether gain-of-function is good definition or not. That began with the NIH. They gave us the definition and we started with that. I do think Dr. Esvelt is making some good points that we ought to be concerned with viruses that are not created but that actually come from nature that could cause pandemics. I think part of this discussion is to try to figure out where we get to.

Senator Johnson.

OPENING STATEMENT OF SENATOR JOHNSON

Senator JOHNSON. Thank you, Mr. Chairman. How long have we had gain-of-function capability? Is that with the CRISPR technology? Mr. Esvelt.

Mr. ESVELT. I should probably defer to Dr. Ebright on that.

Senator JOHNSON. Dr. Ebright, how long have we even had this capability?

Mr. EBRIGHT. The discussions have been underway since 2002 and 2003. The first examples involved reconstruction of previously eradicated or extinct pathogens. Those presented a prototype for understanding experiments that would create new health threats and the need to address them. Again, we are talking a two-decade-long discussion.

Senator JOHNSON. The technology emerged or they started discussing it and then developed the technology—which came first?

Mr. EBRIGHT. The discussions occurred as the technology emerged. It became possible to do this effectively, starting at the beginning of the millennium. The technologies have increased in sophistication and have increased in ease and decreased in cost over time.

Senator JOHNSON. Talk about the ease and the cost because I have heard it is very accessible now and it is very cheap, and a knowledgeable individual can basically do this in their garage.

Mr. EBRIGHT. That is an exaggeration. But as Dr. Esvelt has pointed out, given the genome sequence of a virus it is typically possible to reconstruct infectious particles of the virus and to do so for costs well under $10,000 in one-person month or two-person months. For an equipped laboratory, the kind of laboratory that would be present in any State program, and that is present in many research laboratories at academic institutions, this is eminently possible.

Senator JOHNSON. Reconstructing a virus is one thing, but my understanding of what, at least, the theory might be with severe acute respiratory syndrome coronavirus (SARS–CoV–2) is there is gene splicing that occurred here and some very unusual markers in this furin cleavage site and it would be beyond my comprehension exactly what that means. But talk to me a little bit about the whole gene-splicing aspect of this.

Mr. ESVELT. There are two ways to edit a virus. Nowadays the easiest way is usually to assemble it from scratch using synthetic DNA. But if it large then in some cases it is better to create the altered piece that you wish to insert into the virus and then use a tool such as CRISPR to do the insertion into the backbone.

With respect to the cost, the first virus with a chemically synthesized genome from synthetic DNA was made in 2002. Since then, the cost of gene synthesis has fallen by roughly 1,000-fold. Today the cost of ordering the components of an infectious influenza virus, for example, the synthetic DNA costs less than $1,000, and that does not require any further editing. That requires following the reverse genetics protocol, transfecting it into the cells to get the infectious virus.

I estimate that there are around 30,000 people who can do that, who have doctorates, and you can say 125 virology Ph.D.s per year are in the United States. That is roughly one-third in the world. There are probably four times as many people who have degrees in other disciplines, such as mine, who can do it. Assume a 20-year career, and that is 30,000 people, add a few technicians.

Senator JOHNSON. Was there a specific incidence or something that concerned people that caused the Pause?

Dr. QUAY. Yes. There were experiments in influenza in the Netherlands and Wisconsin that took a virus that was 90 percent lethal but not airborne and created it and made it airborne through passage in the laboratory.

Senator JOHNSON. That occurred when?

Dr. QUAY. In 2013, 2012.

Senator JOHNSON. That caught the attention of who? I mean, who was alarmed by that and instituted the Pause? I know it had to have occurred under President Obama, but which member of our health agencies?

Dr. QUAY. I think Dr. Ebright would be the best to answer that.

Senator JOHNSON. Dr. Ebright.

Mr. EBRIGHT. The proximal impetus for the Pause was a series of events, laboratory accidents at Federal laboratories that have access to and storage of potential pandemic pathogens. The accidents

included an anthrax incident at the CDC, another anthrax incident at a U.S. Army facility at Dugway in Utah, and the finding of unsecured vials labeled "smallpox virus" in an FDA NIH freezer in Maryland. Those three incidents, occurring in close succession, resulted in a hearing in the House Energy and Commerce Committee and then action by the Office of Science, Technology, and Policy. The Pause was driven, ultimately, from the White House, from the Obama Office of Science, Technology, and Policy.

Senator JOHNSON. Listening to your testimony I am assuming all three of you would agree with this statement that this research—and I would say even the mining of dangerous potential pathogens, crawl in a bat cave and try and pull these things out and bring them to a lab—there is surely no benefit that overrides the risk. We should not be doing this at all.

Dr. QUAY. Yes. I call it gain-of-function and gain-of-opportunity, where you bring a virus back. As I said, my analysis is that it has not contributed to the response to this pandemic.

Senator JOHNSON. We should not do it. I mean, we can talk about controls but the bottom line, we should not have controls so we should not even do it. Is that your position as well?

Mr. ESVELT. For balancing the potential benefits of prevention against the risk of accidents it can go either way, depending on the numbers you use for those. You can reasonably come out with either answer. When you add the misuse case, that is what absolutely blows it out of the water.

Senator JOHNSON. Dr. Ebright.

Mr. EBRIGHT. I believe a strong case can be made, or a case can be made that certain components of gain-of-function research of concern, particularly components involving pathogens that are currently in human populations, are categorically separate and more justifiable than other components of gain-of-function research of concern.

For example, currently SARS–CoV–2, the virus responsible for COVID, is present in millions of humans and is generating variant after variant. Gain-of-function research of concern on SARS–CoV–2 involving the creation of new variants and analysis of the threat posed by them arguably can be justified because this is not creating new health threats that will not arise without intervention but is addressing a health threat that is in place currently.

For that reason and for reasons like that, I believe enhancing the oversight of the research is more a more effective and more prudent strategy than simply banning it.

Senator JOHNSON. I would say improved oversight but would you also agree dramatically limit it?

Mr. EBRIGHT. Absolutely.

Senator JOHNSON. OK. Thank you, Mr. Chairman.

Senator PAUL. Senator Marshall.

OPENING STATEMENT OF SENATOR MARSHALL

Senator MARSHALL. Thank you, Mr. Chairman, and I hope America is listening today. To our witnesses let me say welcome, and I regret that none of you were able to get into the Kansas State University biochemistry program, but I certainly appreciate your credentials that are all here today.

I think it is important to not only identify the true problem but also talk about where we have been, and you all can help us fill in some of the pieces here when we talk about gain-of-function research.

It was late in 2011, when the National Science Advisory Board on Biosecurity (NSABB), which is the NIH's advisory board, stopped two scientists from publishing an influenza gain-of-function study that I believe Dr. Ebright was referring to. They stopped it because they were afraid it could afraid bioterrorists. This is 2011. Over a decade ago, scientists had figured out how to make H5N1, which is highly pathogenic avian influenza, more contagious.

In 2012, those 2 scientists and 39 others implemented a voluntary gain-of-function research pause on influenza experiments. In early 2012, Dr. Fauci encouraged all influenza scientists to pause gain-of-function, and said, and I am quoting Dr. Fauci, 2012, "It is essential we respect the concern of the public, domestically or globally, and not ask them to take the word of the influenza scientists." It is interesting to me that Dr. Fauci was focused on the messaging but he still wanted to continue the gain-of-function research.

Again, in 2012, Dr. Fauci also said, almost prophetically, that he worried about unregulated laboratories, perhaps outside the United States, doing work sloppily and leading to an inadvertent pandemic. He went on to say the accidental release is what the world is really worried about.

I go forward to 2014 now, after biosecurity accidents in United States research labs, which our witnesses have talked about, the Obama White House implemented the second gain-of-function moratorium on influenza plus MERS and SARS because of the potential risk of lab accidents and inherent gain-of-function danger. But gain-of-function still continued at the University of North Carolina, research later that we shared with Dr. Shi, the Bat Lady.

Nevertheless, clearly the U.S. Government and Dr. Fauci knew that the viral gain-of-function research was very concerning. Almost counterintuitively, while Dr. Fauci encouraged United States scientists to pause their GOF studies, Dr. Fauci offshored the paused research to China, not once but twice. In 2012, Dr. Fauci gave a new grant to Peter Daszak's EcoHealth Alliance for influenza research in China, and then again in 2014, Dr. Fauci gave another grant to Daszak for SARS research in China. Daszak partnered with who? The Wuhan Institute of Virology.

In late 2017, NIH announced a lift on the gain-of-function moratorium, what became known as the P3CO Framework, that we referred to, apparently without consultation from a Senate-confirmed State Department head or national security leadership. Also significant, there was no Office of Science and Technology Policy (OSTP) director in place and only an acting HHS Secretary at the helm.

What was the result of this? NIH essentially lifts the moratorium on their own by slipping it in-between administrations and self-policing. Today we cannot see the research record for Dr. Fauci's offshore projects because the Chinese Communist Party supposedly has EcoHealth's records, and NIH resists sharing theirs.

I will get to my question now. Dr. Ebright, could EcoHealth research in China have led to the COVID–19 pandemic and Dr. Fauci's worst fears that a lab accident in a foreign lab became reality?

Mr. EBRIGHT. Yes. Lapses in U.S. oversight of gain-of-function research of concern may have caused the current pandemic, and could cause future pandemics. The U.S. Government funded high-risk gain-of-function research and high-risk enhanced potential pandemic pathogens research at the Wuhan Institute of Virology in 2016 to 2019. The research overlapped the pause that was in effect in 2014 to 2017, and met the criteria to be paused, but was not paused.

The research also overlapped the subsequent policy, the P3CO Framework, that has been in effect from 2018 to the present, and met the criteria for Federal risk benefit review under the P3CO Framework, but did not undergo Federal risk benefit review under the P3CO Framework.

Senator MARSHALL. Thank you so much. I have to stop and point out, too, that USAID, who is knee-deep in this type of research, is part of the State Department, where they can get the security advice that they should have asked for before they cleared this with P3CO.

Certainly I believe that this virus came from Wuhan, China, and that it is a product of gain-of-function research. This is a bipartisan national security issue, like several of our witnesses have testified, that this viral gain-of-function could become, and has become a weapon of mass destruction, that this model—this is a 3–D model of what the COVID virus looks like, and this is the gain-of-function. This is the protein spike, the two units that allows this key to fit into the door perfectly and the cleavage site and all that. This became a nuclear hand grenade, is what happened.

Dr. Quay then Dr. Esvelt, considering the extreme risk of this research and the incredulous obstruction by the NIH, USAID, EcoHealth, and China, should Congress immediately pause this dangerous research?

Dr. QUAY. I think that is an appropriate step for Congress to take.

Senator MARSHALL. OK. Dr. Esvelt.

Mr. ESVELT. I think it would be somewhat dangerous to attempt to pause gain-of-function research when it is evident that that term is so malleable as to be evaded at will, and also could plausibly do damage by applying to science that is not specifically directed at potential pandemic pathogens.

Senator MARSHALL. Are there any countries that you would say we should not be doing this type of research with?

Mr. ESVELT. When it comes to identifying pandemic-capable viruses that could kill millions of people and will necessarily be shared with scientists worldwide who will be able to access them, I do not think that we should be doing it. I do not think that China should be doing it. I do not think that anyone should be doing it, because it is expected to kill 100 times as many people as it might save, even if we could perfectly prevent an identified natural virus from spilling over.

Senator MARSHALL. Thank you, Mr. Chairman. I have some more questions if we have time for later, but I yield the floor back. Thank you.

Senator PAUL. Senator Hawley.

OPENING STATEMENT OF SENATOR HAWLEY

Senator HAWLEY. Thank you, Mr. Chairman. Thanks to the witnesses for being here.

Dr. Quay, if I could start with you. You said in your written testimony that the genome of COVID has some of the hallmarks of gain-of-function research, and in particular three genomic regions you say have the signature of synthetic biology. One region has features of the two types of forbidden gain-of-function research that are associated with bioweapons development. You said in your opening remarks that you believe COVID–19 was the product of gain-of-function research and was from a lab leak from the Wuhan Institute of Virology.

My question, I guess, is, do you think China engaged in a cover-up to prevent the world from knowing the true origins of this virus and a lab leak?

Dr. QUAY. I think there is abundant evidence that they have not shared all the information they had at the time. They continue to not share information. I could give you a laundry list of 20 things that they have done, starting with a website with 21,000 viruses. On September 12th at 2 a.m., someone was in the Wuhan Institute of Virology. That had been available to virologists for a decade. It was taken offline. It has not been returned. We have asked to see it, and no one, that I know of, has ever seen it. It goes on from there.

Senator HAWLEY. Are you concerned with the continuation and expansion of Chinese gain-of-function research?

Dr. QUAY. I think I testified here that in December 2019, they were doing synthetic biology on a cloning vector of the Nipah virus, which is 60 percent lethal. We just experienced a 1 percent lethal virus. My estimates would be that that could set us back a millennium. The black plague was a 20 percent lethal event and it was 250 years for civilization to return.

Senator HAWLEY. Let me ask you this. How safe were the testing conditions at Wuhan, to your knowledge?

Dr. QUAY. I think that a lot of the Western virologists actually use the findings of that as a way to get around saying it was OK at the beginning. All of the work that I have described is being done at what is called BSL–2, 3 level, which is commonly spoken of as a dentist's laboratory level of biosafety. Maybe a little higher than that, but that is not a bad euphemism.

Senator HAWLEY. You said, I think, in your testimony, that this is the most dangerous research that you have ever encountered. What makes this particular research so dangerous?

Dr. QUAY. If you doing experiments with a pathogen that is 60 percent lethal but is not airborne, and you make it airborne in the laboratory and someone walks out with it—Nipah has a 21-day incubation period. It is perfect for wide spread without being detected. We cannot afford 10 percent lethality.

Senator HAWLEY. Yes. Dr. Ebright, let me ask you about the merits of gain-of-function research because I was struck by something you said in your written testimony. You said gain-of-function research has no civilian practical applications. From a research perspective, then, why do it? I mean, what is the value, the real value of gain-of-function research?

Mr. EBRIGHT. Not a matter of value but incentives, particularly incentives within the academic research ecosystem. Gain-of-function research of concern is fast and easy, much faster and much easier than vaccine or drug development. Gain-of-function research is publishable and gain-of-function research is fundable. With those four incentives in place—fast, easy, fundable, and publishable—the research will be performed. Eliminate any one of those incentives and it will not be.

Senator HAWLEY. Thinking about China for a second, what is China's interest in gain-of-function research?

Mr. EBRIGHT. They have witnessed the United States leading the way with gain-of-function research. Most gain-of-function research of concern performed to date has been performed either in the United States, with U.S. funding, or overseas with U.S. funding. China has wished to be part of that and has participated in gain-of-function research of concern in China with U.S. funding and has also supported gain-of-function research of concern in China entirely through Chinese programs.

Senator HAWLEY. Let me ask you this. Gain-of-function research and bioweapons, what is the connection there? I mean, what role does gain-of-function research play?

Mr. EBRIGHT. As I mentioned, there are no civilian practical applications. There are immense bioweapons practical applications. As you have heard from Dr. Esvelt, the potential pandemic pathogens that can emerge from such studies are potential weapons of mass destruction—inexpensive, accessible, easily distributed weapons of mass destruction.

Senator HAWLEY. Let me ask you about some of the things that you have commented on with regard to what NIH and Dr. Fauci have said, and frankly, the lies they have been caught in regarding the coronavirus. I want to highlight two of them.

In response to a congressional inquiry from October 2021, just last year, the NIH attempted to walk back assertions by NIH Director Collins and Fauci that NIH had not funded gain-of-function research in Wuhan. You commented at the time, saying, and I am going to quote you now, "NIH, specifically Collins, Fauci, and Daszak lied to Congress, lied to the press, and lied to the public, knowingly, willfully, brazenly. On May 11th, Dr. Fauci said the NIH and National Institute of Allergy and Infectious Disease (NIAID) categorically has not funded gain-of-function research to be conducted in the Wuhan Institute of Virology." You commented on that, saying the documents make it clear that assertions by the NIH Director, Francis Collins, and Fauci, that the NIH did not support gain-of-function research are untruthful.

Expand on that if you would. What are the implications of Dr. Fauci's continued blatant dishonesty regarding NIH's funding of gain-of-function research in Wuhan?

Mr. EBRIGHT. I stand by my statement. The statements made on repeated occasions to the public, to the press, and to policymakers by the NAIAD Director, Dr. Fauci, have been untruthful. I do not understand why those statements are being made because they are demonstrably false.

Senator HAWLEY. In my few remaining seconds here, let me ask you about an effort to shut down any kind of questioning of the origins of COVID. On February 19, 2020, a group of virologists and others published that famous letter, infamous letter, in The Lancet, which said, among other things, "We stand together to strongly condemn conspiracy theories suggesting COVID–19 does not have a natural origin."

Of course, we later found out that The Lancet letter had been organized by Peter Daszak, president of EcoHealth Alliance, who we have discussed today operated a lab in Wuhan, with a $600,000, 5-year annual grant of taxpayer dollars from Fauci's NAIAD to study bat coronaviruses.

That letter conveniently concluded by stating, "We declare no competing interests." Many people designate this letter as the first effort to quash any kind of debate about the origins of COVID–19. Do you think that labeling the lab leak theory as a conspiracy theory so early on have the effect of slowing down investigations into the origins of the virus?

Mr. EBRIGHT. It certainly had that effect, but The Lancet letter that you described was only one of two efforts to impose the false narrative that science shows SARS–CoV–2 entered humans through natural spillover, and that that is the consensus view of scientists. One of the efforts was The Lancet letter you discussed. The other effort was coordinated and orchestrated through the National Institutes of Health, through the NAIAD Director, Dr. Fauci, and the NIH Director, Dr. Collins, and resulted in the publication of an opinion article entitled "Proximal Origins of SARS–CoV–2," making the case, again, that SARS–CoV–2 could not have been a product of a research-related spillover."

Senator HAWLEY. Thank you very much. Thank you, Mr. Chairman.

Senator PAUL. Thank you. Had there not been a pandemic I think there would still be a need for this hearing. This discussion, Dr. Ebright got this started back as early as 2003, 2004. Others have commented on the danger of being able to manipulate influenza viruses to be used as either weapons or by accidental release.

But I think given that there was this pandemic, that a million Americans died, I lost friends, good friends, to the pandemic—I think we should be curious. I am perplexed by the lack of curiosity to know are there any precautions we can take, is there any kind of government oversight that we could do to try to prevent this from happening.

Now some will say, we cannot prove it came from a lab. That is, in all possibility, true, that we cannot prove it. But there are arguments to be made and examination of facts to give us an idea of whether it might have come from a lab. Even if we did not, I think that this could have come from a person in a lab handling a virus, if it was a virus out of nature, and we have discussed that as well.

I do think that we have to get to the truth of the matter of whether or not dangerous research was going on that should have been reviewable. We had a pause of gain-of-function research, but then we had research occurring during the pause that should have gone to this committee, this P3CO committee, and did not get to the committee.

I think Dr. Ebright described it well. He says that in Wuhan, in the 2016 to 2018 period, they were constructing novel chimeric SARS-related coronaviruses that combined the spike gene of bat SARS coronavirus with the rest of the genetic information of a SARS1-related virus, one that was already known to have lethality, and they found that it could efficiently infect human airway cells and exhibited up to a 10,000-fold increase in viral growth.

But when we have asked before, is this gain-of-function, we get sort of arguments and protestations that this is not gain-of-function as if this is no big deal and the experts looked at this. As we look farther into this we find that the experts never looked at this, that it is sort of a select-in kind of program to this committee. It does not go looking for dangerous research. It looks at it if you come to them and say, "Hey, I think I have gain-of-function research. Do you all want to look at my research?" And so there is this opting-in aspect to this.

But I think it is important that we get to the truth. Was there research going on in Wuhan that was dangerous? Was it funded by the NIH, and should it have gone through this committee process?

By the definition that they have given us, gain-of-function—I think I agree with Dr. Esvelt—can be better defined, and particularly if we are going to have oversight on this we are going to have to figure out what our oversight is going to be. By all means moving forward we need to ask and include the scientists to get a precise definition of what we are talking about if we want to have more oversight.

We have to look back before we look forward, not so much to assign blame but to figure out is it really necessary. Do we need to have hearings on this? Should we have follow-up hearings? Should we have legislation? If a million people died and there is a chance this came from a lab, I think without question we should. Both sides of the aisle should be looking at this.

My question, and I think it is pretty clear but I would like to go through everybody, even though Dr. Ebright has said this was gain-of-function, to each of the three witnesses, was the research, where you take the backbone of a SARS1 virus that has known lethality, and you mix it together with an unknown bat virus, S protein genes to create a new virus, was this gain-of-function according to the NIH definition and should it have been reviewed and discussed by this committee that was supposed to prevent dangerous research from going on?

We will start with Dr. Ebright.

Mr. EBRIGHT. As you mentioned, the Wuhan Institute of Virology constructed novel chimeric SARS-related coronaviruses that combined the spike gene of one coronavirus with the genetic information of another. They showed that the resulting viruses efficiently infected human airway cells and efficiently replicated in human airway cells, and they showed that the resulting viruses exhibited

up to 10,000-fold enhancement of viral growth in lungs and up to 4-fold enhancement of lethality in mice engineered to display human receptors on airway cells.

Based on those facts, and they are, indeed facts, the research was gain-of-function research of concern subject to the Pause, and was enhanced potential pandemic pathogen research subject to the P3CO Framework. Nevertheless, due to the failure of the NIH to forward the proposals for review, the work was not paused and there was no P3CO review.

Senator PAUL. Dr. Quay.

Dr. QUAY. The Wuhan Institute of Virology is unique in the entire world. Before 2019, 65 percent of all publications on coronaviruses came from that single institution. They are unique for two reasons. For almost a decade, they were going into bat caves throughout China and actually into Africa as well, 20 visits a year, and bringing these samples back to the laboratory.

On the one hand they had the largest collection of raw material backbones from nature to then do gain-of-function research on. They trained in Galveston, Texas, and in North Carolina, and were doing experiments, published experiments between 2015 and 2019.

I believe it is the confluence of those two activities, gain-of opportunity, bringing things back from bat caves, and gain-of-function research, that led to the pandemic.

Senator PAUL. Dr. Esvelt.

Mr. ESVELT. On the list of experiments you would need to perform in order to learn whether a novel virus could potentially cause a pandemic you would need to test growth in human primary cells, such as human airway epithelial cells, and you would need to test transmission in a suitable animal model.

The question is, if they were not intending to determine whether a novel recombinant event between these coronaviruses could lead to something that might kill millions of people then why were they doing it? If there was no chance that it would come up with a result that looked like it was more dangerous, what is the point? What is the scientific hypothesis?

Again, whatever you call it, what they were trying to do was identify a biological agent that has a good chance of being able to kill millions of people if released. They shared the description of what they did and they shared the genome sequence, because they thought that this would make us safer, because they think that knowing which viruses in nature might cause pandemics makes us safer.

They did not consider the security risks, and it is worth noting that both USAID and NIH funded those particular coronavirus chimeric studies. USAID, to my understanding, has since disavowed those chimeric recombination studies and announced that they will only focus on finding natural pandemic-capable viruses, which is at least a step in the right direction. But again, I would call that gain-of-function. Another reasonable scientist would say, no, that is not gain-of-function, because the term is so ill-defined.

Senator PAUL. Even beyond the term, though, would it be qualified as dangerous research that actually should have gone before this committee, the P3CO committee, and been reviewed?

Mr. ESVELT. Here is where you come back to the problem of thinking this is a health and safety issue rather than a national security issue. The question is why are we trying to identify readily accessible agents that could plausibly be used to kill millions, and will, as soon as identified, fall into the hands of all of our adversaries as well as, perhaps, individual terrorists who would want to use them?

The fundamental principle behind even wanting to do these experiments in the first place is, I think, a fundamental threat to not just national security but international security. It is hard to see why you would ever want to do this, when you think about the misuse potential. I have not seen anyone else publish a numerical model of that.

Senator PAUL. People have said, well, the closest relative that we have found is only 96 percent identical to COVID–19. This could not have come from the lab. They have also mistakenly accused those who say it came from the lab saying, oh, it came from this particular variant. I think what people who are saying that this could have come from a lab are saying is that there could also be possibly other viruses that are closer that were manipulated or that the one that is 96 percent analogous to COVID–19 could have gone through serial cell culture and become COVID–19.

I would like to ask the three of you whether or not the variant that is 96 percent analogous to COVID–19, could it, through serial passage, be transformed to COVID–19? Is it possible? Is it so far away that you cannot do it experimentally? Could you do it through gene splicing? Could it be done? Or is it something that argues that this could not have come from the lab?

We will start with Dr. Ebright.

Mr. EBRIGHT. The closest relatives are more on the order of 97 percent identical to SARS–CoV–2 genome than 96 percent. Viruses with that level of genetic difference cannot rapidly, in the time scale of weeks or months, move from their State into being a proximal progenitor of SARS–CoV–2. However, in the laboratory those viruses can be combined, at will.

They can be combined, in particular, using a method that would be described as constructing a consensus genome virus. In a constructed consensus genome virus, one takes the sequences of several related viruses, identifies the most commonly observed nucleotides at each position in these sequences, and then synthesizes the nucleic acid corresponding to the average, if you will, the consensus genome for the group of viruses.

This has been done successfully in coronaviruses. This has been done and published a decade ago in coronaviruses. That kind of research could have been done using viruses that are on the order of 96 to 97 percent identical in their genome sequences to SARS–CoV–2 and with two or three or more such virus genome sequences, one could develop a consensus.

That is just 1 of a series of potential routes by which one of the known viruses with 96 to 97 percent identity could, through a laboratory, in a relatively short time, be transformed into a progenitor of SARS–CoV–2.

Senator PAUL. Dr. Quay.

Dr. QUAY. The three sets of viruses that are closest to SARS2 are one from southern China, RTG–13, and a series of BANAL from northern Laos. As indicated there are probably 1,200 letters different in the whole 30,000-letter alphabet. In nature, that takes approximately 40 years, so the most common ancestor is about 40 years ago. But most of that can be done in a couple days in a laboratory.

However, I do not believe we currently have the starting material, the backbone on which SARS2 was found. I think it is one of the other 21,000 viruses in the database that was taken down at 2 a.m., September 12, 2019.

Senator PAUL. A great deal of information was destroyed by the Chinese.

Dr. QUAY. It was taken offline and not available. I do not know if it was destroyed.

Senator PAUL. Dr. Esvelt.

Dr. ESVELT. If a Ph.D. student proposed to take a 30,000-base-paired viral genome and attempt to passage it in the laboratory to acquire 1,000 or so mutations, I would say that is not a Ph.D. project. Go do something else. I concur with Dr. Ebright that the only way that you could get something so divergent would be to computationally design it and synthesize it, which could certainly have been done, from what dataset, and again, why? Why would you do such a thing unless you want to know what the ancestral virus was like and whether the ancestral virus was dangerous. There are basic science reasons why you might want to know where they all came from, but at the end of the day the reason why this research is of interest to us is the risk of pandemics.

Again, why would you run the tests to determine whether something was pandemic capable? They certainly ran those on all of the other coronaviruses that they found and thought might be dangerous. On the other hand, they never published anything like that, right, and presumably they would have. They published their data on the other stuff.

This is why I do not think we have enough information to know, but it was definitely not passaged in a lab from something that was maybe 7 percent——

Senator PAUL. I agree, and one of the things that tips us off that they may have been trying was in 2018, they asked for money from Defense Advanced Research Projects Agency (DARPA), and in that money they wanted to insert the furin cleavage site, which makes it highly infectious in humans. If they had the idea of that and they are asking for money, they must have thought, wow, we can do this and this is going to be a great experiment. Even our government, finally, at that point, decided not to fund that.

But what they are asking for, and this is why I think there was a "holy cow" moment when all of a sudden these scientists see the sequence of COVID–19, they say, "Oh, my goodness. Didn't they ask us, in 2018, to put that furin cleavage site in?" Lo and behold, it is there.

What I am going to ask, and I am going to finish with this and then we will have another round if some people would like to ask some other questions, is, Dr. Quay, could you sort of lay out, in as simple a fashion as possible, two or three items about the virus

that makes you think it came from—and I do not think anybody knows, with 100 percent, whether this came from a lab or whether it came from animals, but if there is some compelling evidence that suggests it could have come from the lab. Even if it was a 10 percent chance it came from a lab it is another reason for us to be concerned about having oversight on this kind of research.

Can you give me two or three things that this virus has that makes you think it is from a lab versus some of the evidence for MERS and SARS that it came from animals?

Dr. QUAY. Yes. There are three regions—the receptor binding domain, the furin cleavage site, and this protein 8 from a gene called ORF8. With respect to the receptor binding domain, if you look at what happened with SARS1, we have the virus sequenced when it first was in civet cats in the markets. It jumped into a few humans. We have the virus sequenced then. It started infecting more. Then we have the virus sequenced when human-to-human passage could occur and an epidemic occurred. You can see the progression of mutations as the virus adapted from being in civet cats and then being in humans. The first jump into humans it had only 15 percent of the mutations it needed to support an epidemic.

OK. Let us look to SARS–CoV–2. When you look at the virus that first entered the human population, out of all of the changes in the receptor binding domain there are 200 amino acids, 4,000 possible changes. There were only 17 mutations that could make it a better virus. Its receptor binding optimization was 99.5 percent, and, in fact, one of the 17 ended up being the Delta variant. That kind of optimization, juxtaposed by the fact that there were no patients in Wuhan, 36,000 blood-backed specimens tested for antibodies, not a single patient was infected.

Let us go back to SARS1. Twenty percent of all people in the markets were infected while the virus was practicing to set up an epidemic, 1 percent of the general population. We would have expected 360 in the general population in Wuhan, and we had zero.

Furin cleavage site has obviously never occurred in this related virus, the sarbecoviruses, that split from their cousins, the MERS viruses, around the time of William crossing the Channel, 1060. That was when sarbecoviruses came. There has never been a furin cleavage site, and the genetic sequence of it uses a code that has never been used, the CGG–CGG dimers, it is called, which has never been used before.

Finally, ORF8, this protein that goes into the bloodstream and suppresses interferon response so you are asymptomatic, and suppresses major histocompatibility complex (MHC) antigen presentation, so you cannot make good antibodies. This was the subject of two master's theses at the Wuhan Institute of Virology. I have found no Western scientists that worked on this location in the genome before 2019. The protein is not present in MERS. It has a 5 percent homology in SARS1. Between SARS1 and SARS2 there is a protein there but it is only 5 percent homologous.

But this master's thesis, the first one optimized its function in suppressing interferon, symptoms of fever and chills, and suppressed its antigen presentation. The second one was making synthetic biology tools so you could move it around inside genomes.

Senator PAUL. To reiterate, there have been no animals found that have COVID–19. When they did find that animals had the first SARS and MERS, they found it out within months. When they tested the animals in question, 90 percent of the animals had the SARS virus. We have not found any animals yet with COVID–19. Most viruses that come from animals first are not very infectious at first and they infect a few humans. You do not have a pandemic that does this. It smolders and then does this. During the smoldering phase you find background antibodies that people have had it, even if they do not know they had it.

When they tested the background of people who were working with the animals that had COVID they found 20 percent of them hand antibodies to having had SARS.

Dr. QUAY. SARS1, yes. Correct.

Senator PAUL. But then if we test the people in the marketplace we are not finding that. If we look at the people in the Wuhan marketplace we are not finding significant numbers that were positive, and finding almost nobody positive from the previous year that had been ill.

Dr. QUAY. No. It is zero out of 36,000.

Senator PAUL. Thank you.

Why do we not do a second round, and we will go in the same order. Senator Johnson.

Senator JOHNSON. Dr. Quay, how did we find out about the Nipah virus?

Dr. QUAY. In December 2019, five patients at a Wuhan hospital had their specimens sent—a bronchial lavage, where they stick into the throat and get a specimen—to the Wuhan Institute of Virology for sequencing. The process is to amplify it with a polymerase chain reaction (PCR) process. You make a lot of copies of what is in the specimen and you usually, inadvertently, make copies of what is going on in the laboratory.

The Wuhan Institute of Virology probably regrets, but they put a 55 million-letter database of the background information up in the gene bank, which is the NIH's database there, of everything going on. We found 20 strange things in these patient specimens—honeysuckle genes, horse viruses. Nineteen of the things we found were in publications from the laboratory over the previous 2 years. This clearly was a signal of what was going on in the lab around there.

The one thing they did not publish on was the cloning vectors of the Nipah virus. It is in the patient specimens because it was in the laboratory at the time, not in the patients, and they have never published on that at this point in time.

Senator JOHNSON. How do we know it is 60 percent lethal?

Dr. QUAY. The Nipah has had epidemics, sporadic epidemics in the belt around Africa and India, Bangladesh, and it is between 60 and 80 percent lethal in the pockets where it comes out. It is not very transmissible like Ebola so it kills 100 or 200 people and then burns out. But if they made it airborne it would be different.

Senator JOHNSON. OK. This is a virus that occurs in nature but you detected it in this database.

Dr. QUAY. I detected cloning vectors of it. They are manipulating it, which is not allowed by biological treaties.

Senator JOHNSON. That is a pretty scary scenario right there, that the Wuhan lab that might have been the originator of the coronavirus is fooling around with something far more deadly.

Dr. QUAY. Yes.

Senator JOHNSON. Obviously mum is the word.

Dr. Ebright, I am a little confused. You talked about, if we were doing gain-of-function on the current coronavirus that would be OK. That is not the indication I am getting from Dr. Esvelt here. The thing that really concerns me is—and I am not saying that you are saying this is the justification. You are just saying the reality situation is we have research centers, we have scientists that are doing this gain-of-function research, I mean very dangerous gain-of-function research, for two completely unnecessary reasons, because it is fundable and it is publishable. You have a little greed involved and you have hubris. Is that what you are saying?

Mr. EBRIGHT. The research is performed because it is fast, easy, fundable, and publishable. In the academic research ecosystem those are determinants of what research gets pursued.

Senator JOHNSON. I view that as a very corrupt research ecosystem. If that is what is driving research, and very dangerous research, it is so that you can get a funding grant just to do something for grins and then he can publish it and get the academic kudos for it. I am sorry. I just find that sick.

Mr. EBRIGHT. I would not use the term corrupt. I would not see any real difference between this than the activity of a hedge fund or the activity of a bank or a broker. The key point is that because of these incentives, self-regulation from within the community is insufficient. The scientific research community will follow the incentives. It will never effectively self-regulate on these issues.

For this reason, we have regulations with force of law for vertebrate animals research and for human subjects research. We need regulations with force of law for gain-of-function research of concern.

Senator JOHNSON. I think the difference, if it is a bank or hedge fund, they are doing things for an economic incentive, to produce something to fund a manufacturing site or fund some kind of business. I am not hearing the benefit of this research. I am seeing the risk. I am seeing the danger. I am not seeing the benefit, other than what you are saying, for the researcher itself to get money, to do something that is dangerous, and have the academic kudos for being published.

I do not know. Maybe you do not like the word "corrupt." It is completely useless. It has no benefit to society. It just has risk. It just has danger.

Dr. Esvelt, do you disagree with that assessment?

Mr. ESVELT. I think that all institutions follow their incentives, and I think that set of incentives—fast, easy, fundable, and publishable—insofar as fundable and publishable are ways of curing heart disease and cancer and forestalling aging, those are all certainly fundable and publishable, perhaps not as fundable as we would like. Certainly research into defenses against the next pandemic is right now somewhat fundable. I wish it could be more fundable. It is publishable, right? It depends on——

Senator JOHNSON. What you are talking about fundable and publishable have a beneficial reason. What I am hearing from the three of you witnesses, there is just not a benefit to this.

Mr. ESVELT. One clarification. You mentioned on endemic human viruses like SARS2, why do this. If you want to predict the next variant that is going to arise anyway, within a couple of months, one that already exists, then that is why researchers do things like deep mutational scanning of the spike protein to look and see which ones of them might have a bit of an edge in terms of maintaining infection while evading immunity a little bit, and is likely to maybe be the next variant. That then lets us design the next vaccine against the variant and guess correctly.

We have to do this with flu every year. Flu vaccines are terrible, usually, because we often guess wrong. That kind of research can help improve our guess as to what is correct.

But as soon as you make a change that would not occur in nature, then it becomes dangerous because that is something that a more pathogenic mutation could be inserted. That becomes a problem and there is no justification for doing that because nature is not going to come up with it.

Senator JOHNSON. OK. Thank you, Mr. Chairman.

Senator PAUL. Senator Marshall.

Senator MARSHALL. Thank you, Mr. Chairman, and thank you again to our witnesses for hanging in there with us.

I want to start by going back to a comment that Dr. Esvelt made, that USAID paid for gain-of-function research in China. Most people do not realize that because USAID will not give us the records, and we have been trying for over a year to get those records, which is why we are holding up one of their nominees as well. Thank you for pointing that out, Dr. Esvelt.

I am going to go to Dr. Ebright next and talk a little bit more about EcoHealth Alliance, about their record of noncompliance. They could not provide research records to NIH when NIH requested them. They did not have an adequate agreement with Wuhan Institute of Virology. They do not use appropriate rate of pay for researchers. There continues to be noncompliance with financial conflicts of interest policies.

Dr. Ebright, based upon EcoHealth Alliance's record of noncompliance, should they continue to be eligible to receive Federal funds?

Mr. EBRIGHT. Their most important aspect of noncompliance was that they were informed by the NIH, in terms and conditions in the notice of award for their grant, that in the event they encounter viral growth in their engineered coronaviruses that exceeded the growth of the parent coronaviruses by more than a factor of 10, they must immediately inform NIH and immediately stop the research. They did not do this.

That is not merely a financial violation. That is a serious hazard violation and a violation that may be connected to the origins of the current pandemic.

With that being said, it is inexplicable that they were awarded subsequent Federal awards and that they remain eligible to receive Federal awards.

Senator MARSHALL. I need to submit for the record—thank you for the answer—a couple of articles. The first, I quoted Dr. Fauci. This is an article from Science, July 2012. A handsome, young Dr. Fauci. I want to submit that for the record.[1]

My next two questions I want to submit something from The Wall Street Journal, a couple of articles as well regarding genome sequences.[2]

Senator PAUL. Without objection.

Senator MARSHALL. We will go to Dr. Quay next. You may be familiar with the genomic sequences in NIH's database—I think you spoke about them—that Chinese scientists asked to be removed and how they were, from early COVID Wuhan patients. Do you believe there could have been more data in NIH's database submitted by Chinese scientists that could hold a key to the COVID–19 origins?

Dr. QUAY. Yes. This was a really nice piece of work by Jesse Bloom at the University of Washington, who found not in the NIH database but on some Amazon web servers the actual sequences of viruses from very early patients that had been put on GenBank and then removed before they were published and made available.

The remarkable thing is, again, going to another piece of good research, the virus that first came out, the first Wuhan virus, is three mutations away from what we now know is probably the first virus, but that is a computational method. It is kind of complicated. But anyway, there is a prediction. There are three mutations that have never been seen in humans before the first virus that we have in humans. The specimens Jesse found had some of those.

We know that the Chinese have viral sequences that are ancestral to what we have, and the more of those we get, the more we will get to the bottom of this.

I will point out that these sequences were from September and October 2019, 2 months before any person in the market was sick. Again, the timing of the market spillover does not coincide with the genetics of the virus.

Senator MARSHALL. Dr. Esvelt, anything to add to that?

Mr. ESVELT. No, other than Jesse is certainly one of the foremost experts in this field, and if you want probably some of the best answers that science can give then I would recommend that you request his input.

Senator MARSHALL. Thank you. My last question. For 20 years, NIH sponsored EcoHealth's partnership with scientists from the Wuhan Institute of Virology. The Chinese scientists have bragged that their virus sample database is the largest in the world.

They took that database offline in September 2019. NIH asked EcoHealth for research records. EcoHealth told them that the records are in the custody of the Chinese government. Is it possible that the database taken offline by the Chinese government was data collected by EcoHealth and belongs to American taxpayers? Dr. Quay.

Dr. QUAY. Since the work has been funded, in part, by U.S. taxpayers, then by definition access to that would be important. I also

[1] The document submitted by Senator Marshall appears in the Appendix on page 1443.
[2] The document submitted by Senator Marshall appears in the Appendix on page 1446.

think that we do not have to rely on the Wuhan Institute of Virology for releasing that. I believe within U.S. jurisdiction there will be copies of that database. It is too valuable not to have in your own possession if you are doing research on it.

Senator MARSHALL. Do you think there is any way we can still get any of that data that is missing? I feel like, somewhere we are going to find the grandfather of COVID, or the cousin or something here in these data banks.

Why did they take them down? What is the advantage of them taking them down? Do you think we can ever find what we are missing?

Dr. QUAY. It was taken down at 2 a.m. on September 12, 2019, which is—I guess everyone works hard but that is a little suspicious to be doing it at that point in time.

I believe it contains closer precursors, and my hypothesis is it contains the one that is 50 mutations or 100 mutations, not 1,200 away, and it was too obviously a smoking gun.

But again, if you are collaborating on that and you are spending 10 years building a database inside the Wuhan Institute of Virology, you are going to mirror that database in your own facilities, which means that it has to be at EcoHealth Alliance somewhere.

Senator MARSHALL. Thank you. Dr. Esvelt, anything to add?

Mr. ESVELT. Just note that I agree with Dr. Ebright's assessment from earlier, to the extent that China is doing this research, because it is scientifically sexy and glamorous and is fast, easy, publishable, et cetera. Chinese scientists have the same incentives as Western scientists in this regard.

In fact, it is very clear that this research is not in China's strategic interest. China has no more interest than we do in handing out the blueprints to agents that can kill millions of people, including their people. This is not in the interest of any established, powerful nation. The question is, can we show leadership and persuade them of that?

Because as long as we are doing it, we are making it—we are contributing to the fact that this is seen as glamourous research. It gets published in our top-tier journals. Many Chinese scientists get bonuses for publishing in our top-tier journals. We are driving these incentives because we persist in seeing this, again, as a health and safety issue rather than a national security issue.

I think it is in our power to change it, and I think this is one issue where our interests are actually aligned with those of China, and indeed, every other established nation. These are asymmetric tools of mass death.

Senator MARSHALL. OK. Dr. Ebright, anything we did not ask you that we should have?

Mr. EBRIGHT. That I do not know, but I just wanted to agree completely with the last remark by Dr. Esvelt.

Senator MARSHALL. Thank you, and I yield back.

Senator PAUL. I want to thank everybody for being part of this hearing. I do not see this as the end. I see this as the beginning of trying to understand what caused the pandemic and trying to come up with solutions.

Each of your statements, which is longer than your testimony, will be available, for anybody who is interested.

I want to point out one thing from Dr. Ebright's testimony, for those who say, well, lab leaks should be discounted. They do not ever happen.

At one point Dr. Ebright writes, "The second, third, fourth, and fifth entries of the SARS virus"—this was the first one—"into human populations occurred as a laboratory accident in Singapore in 2003, a laboratory accident in Taipei in 2003, and two separate laboratory accidents in Beijing in 2004."

For people who say that it is a conspiracy theory that this could have come from the lab, they are discounting our history. The history has had these lab leaks. Whether or not we will ever know, with 100 percent certainty, whether this came from the lab, we have had lab leaks, and we have to realize the potential danger of these pathogens.

We did not get a great deal of time into the answer. We got a little bit into the answer, but each of the scientists we asked today were asked to let us know how we could better supervise or oversee this kind of research.

The interesting thing to me is I think they all worked independently but they came up with basically very similar solutions, an independent body outside of the funding organizations or those receiving the funding, to make the recommendations, something akin to an independent agency like a nuclear regulatory agency.

In fact, I have already been using the analogy when people ask me and say, "What is this like?" It is essentially we do not let anybody sell centrifuges to Russia or centrifuges to Iran. There are rules on the export of things. I think Dr. Esvelt, in particular, has talked about the security aspect of this.

What I would really like to come of this, and I mean this sincerely, is I would like to have a bipartisan bill that comes forward for better oversight. Maybe it is not oversight of gain-of-function but maybe it includes things that some people consider to be gain-of-function. Maybe it is more general, pandemic viruses. There are a lot of ways we can discuss it.

But the bottom line is I do not think the people doing the research are able to adequately and objectively regulate themselves, and I think having a million people die, there should be bipartisan curiosity in this, that we should be able to move forward.

My hope is that your suggestions, that you have taken the time to put in writing, you have taken the time out of your busy careers to come here, that these suggestions will become legislation. If we can get a bipartisan bill to come forward, what I would like is that our people who help us write the legislation can communicate with the three scientists here. We are willing to hear from a dozen more scientists, anybody who wants to. I want scientists to be involved in this.

But I do think that ultimately the people making the judgment should not be from one small field of science. Some have said, "Well, none of the three scientists there are virologists." I do not have a problem with virologists being part of this, but I do have a problem with them all being virologists, the same way I have a problem with behavioral science being approved for funding by all behavioral scientists. I think that there need to be people who understand science on this, but I think there also needs to be people

on the committee, as Dr. Esvelt as mentioned, that understand bioterrorism and biosecurity.

I think it should be a mixture. This is something we can talk to the scientific community about. I do not think an absolute ban is what we want. What we want is better oversight of this. But we cannot have something where three projects have been looked at in the last 7 years. That means they are not looking.

The fact that they did not look at what went on in Wuhan, and then some of the folks I asked in committee about this were saying, "Oh, our scientists looked at it and approved it," even that is not really true. They did not look at the research. They just ignored the research. It did not go before the committee. They have not been honest.

If we want trust in public health, trust in government, trust in science, trust in research, trust in the NIH, and trust in the grants that we give our universities, billions of dollars, we need to have transparency and honesty. We cannot have a committee where the people are cloaked in secret. I mean, what is this? This is completely insane.

I think we have made some progress. I want to move forward, and I, for one, am open to work with any Democrat in the Senate to make this a bipartisan bill, and to make it an evenly-keeled where all the voices are heard, that we do not rashly create any legislation that would hamper science, but we create something that would have oversight and might save lives.

I truly think that a million people died in our country, six million people died, and I think it was from a lab leak. I think it is something that we need to have precautions against. I think it was accidental, by the way. But I think if we do not do anything, what if this gets in the hands of somebody who actually really wants to harm America or the world, or just some psychopath? What could happen?

Right now we are doing nothing and have changed no behavior. We have had this pandemic and we have changed not one bit of behavior. I think it is about time that we do get together, that we are all curious, and that we do not make this about Republicans and Democrats but make this about how we, as a people, come together to try to make this world a better place.

Thank you all for appearing.

[Whereupon, at 4:13 p.m., the Subcommittee was adjourned.]

APPENDIX

ETSO Subcommittee Hearing: Revisiting Gain of Function Research: What the Pandemic Taught Us and Where Do We Go From Here?

August 3, 2022

Opening Statement of Ranking Member Paul

Good afternoon and welcome to each of our panelists. Thank you for joining us.

The purpose of this hearing by the Subcommittee on Emerging Threats and Spending Oversight is to discuss, as our name implies, the emerging threat posed by gain of function research.

We will hear from a panel of three witnesses, all of whom are extraordinarily accomplished experts in the scientific community. We are grateful for that work, and we are grateful to each of you for taking the time to appear here with us this afternoon.

Gain of function research is a controversial scientific research method involving the manipulation of pathogens to give them a new aspect or ability, such as making viruses more transmissible or dangerous to humans. Despite all we have learned about the potential risks of this particular method of research, this is the first congressional hearing on the subject.

Today we will discuss 1.) what gain of function research entails, 2.) how gain of function research is defined, and 3.) whether the definition of gain of function research is applied consistently by the Department of Health and Human Services P3CO review committee, which is responsible for evaluating the risks & benefits of such research.

We'll also discuss how this P3CO committee operates. The P3CO approves or denies projects from receiving federal funding based on whether the pathogen is considered to be a "credible source of a potential future human pandemic," and if "the potential risks as compared to the potential benefits to society are justified." In other words, a project is not gain of function if the review committee is unsure if a recombinant virus will create a future pandemic. Such broad criterion gives one sole committee, comprised of an unknown group of bureaucrats, the power to spend millions of taxpayer dollars on a single preemptive guess – with potentially devastating consequences.

Today we will also consider whether gain of function research was being performed at the Wuhan Institute of Virology. First, no one – not myself or anyone I'm aware of – argues that a recombinant super-virus that has been published in scientific journals is COVID-19 or a close relative. If COVID-19 leaked from the Wuhan lab, it would be a laboratory-created virus that the Wuhan scientists have not yet, and are unlikely ever, to reveal.

I maintain that the techniques that the NIH funded in Wuhan to create enhanced pathogens may also have been used to create COVID-19. The American people deserve to know how this pandemic started, and to know if the NIH funded research that may have caused this pandemic.

Gain of function research has the potential to unleash a global pandemic that threatens the lives of millions, yet this is only the first time the issue has been discussed in a Congressional committee. I am sure each member of this committee as well as the full Senate can agree that we need stronger government oversight of how our tax dollars are being used to finance experimenting with mutating fatal diseases with outstandingly high mortality rates.

Again, I thank each of our distinguished witnesses for being here today, and I thank Senator Hassan for working with me to convene this hearing.

Chairman Peters Statement for the Record
Subcommittee on Emerging Threats and Spending Oversight Hearing
"Revisiting Gain of Function Research: What the Pandemic Taught Us and Where Do We Go From Here"
August 3, 2022

- This hearing was requested by Ranking Member Rand Paul, and all witnesses were solely picked by the minority.
- There was no involvement by members of the majority.
- In order to ensure the committee record fully reflects the hearing topic, I would like to submit the following documents for the record:
    - Stakeholder Letter on Pathogen Research and Biosecurity, Letter to Honorable Rosa DeLaura, Honorable Kay Granger, Honorable Tom Cole, July 14, 2021.
    - Stakeholder Letter on Pathogen Research and Biosecurity, Letter to Honorable Nancy Pelosi, Honorable Kevin McCarthy, Honorable Chuck Schumer, Honorable Mitch McConnell, April 8, 2022.
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Written Testimony of Richard H. Ebright

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Submitted for the Record to the US Senate Committee on Homeland Security

Subcommittee on Emerging Threats and Spending Oversight

For the Hearing "Revisiting Gain of Function Research"

August 3, 2022

Chair Hassan and members of the Committee:

Thank you for inviting me to discuss gain-of-function research and its oversight. I am Board of Governors Professor of Chemistry and Chemical Biology at Rutgers, The State University of New Jersey, and Laboratory Director at the Waksman Institute of Microbiology. I direct a biomedical research laboratory and serve as project leader on two National Institutes of Health (NIH) research grants. I conduct research on the mechanism of bacterial RNA synthesis and on the development of new antibacterial therapeutic agents able to treat bacterial infections resistant to current drugs. My research involves both priority public health bacterial pathogens (e.g., the pathogens responsible for Staph infections, Strep infections, and tuberculosis) and priority biodefense bacterial pathogens (e.g., the pathogens responsible for anthrax, plague, and tularemia). I am a member of the Institutional Biosafety Committee of Rutgers University, and I have been a member of the Working Group on Pathogen Security of the state of New Jersey, the Controlling Dangerous Pathogens Project of the Center for International Security Studies, and the Biosecurity Advisory Board of the Center for Civilian Biodefense. Here, I discuss the definition of gain-of-function research of concern, risks and benefits of the research, US oversight of the research, and recommended steps to strengthen US oversight of the research. In my written comments, I also include an appendix addressing the origin of SARS-CoV-2 and the possibility that lapses in US oversight of gain-of-function research of concern contributed to the origin of SARS-CoV-2. My assessments are based on information in published NIH, Health and Human Services (HHS), Office of Science and Technology Policy (OSTP), and Congressional Research Service (CRS) documents, on published press reports, on published scientific papers, and on my knowledge of biosafety and biosecurity standards for work with pathogens.

Gain-of-function research of concern

Definition

Gain-of-function research of concern is defined as research activities reasonably anticipated to increase a potential pandemic pathogen's transmissibility, pathogenesis, ability to overcome immune response, or ability to overcome a vaccine or drug. Some definitions also include research activities reasonably anticipated to reconstruct an extinct or eradicated potential pandemic pathogen.

Gain-of-function research of concern involves the creation of *new health threats*--health threats that did not exist previously and that might not have come to exist by natural means for tens, hundreds, thousands, or tens of thousands of years.

Most gain-of-function research of concern to date has been performed in the US with US funding or overseas with US funding.

Gain-of-function research of concern is a small part of the biomedical research enterprise (less than 0.1% of all biomedical research and less than 1% of virology). However, because gain-of-function research of concern can cause pandemics, this small part of the biomedical research enterprise is highly consequential and requires effective oversight.

Risks

Gain-of-function research of concern poses high--potentially existential--risks. Gain-of-function research of concern poses both material risks and information risks.

Gain-of-function research of concern poses *material risks* by creating new or enhanced potential pandemic pathogens. If a resulting new potential pandemic pathogen is released into humans, either by accident or deliberately, this can cause a pandemic.

Gain-of-function research of concern poses *information risks* by providing information on the construction and properties of new potential pandemic pathogens. Publication of the research provides instructions--step-by-step "recipes"--that can be used by a rogue nation, organization, or individual to construct a new potential pandemic pathogen and release it to cause a pandemic. With current biotechnology, the technical means to do this are within the reach of most nations. With improvements in biotechnology in the next decade, the technical means to do this likely also will be within the reach of most sub-state organizations and individuals.

The risks posed by gain-of-function research of concern are *inherent risks*. In some cases, the risks can be mitigated, but in no case can the risks be eliminated.

Benefits

Gain-of-function research of concern provides limited benefits.

Gain-of-function research of concern can advance scientific understanding and, in some cases, can do so more quickly than alternative research strategies.

However, gain-of-function research of concern has no civilian practical applications. In particular, gain-of-function research of concern is not needed for, and does not contribute to, the development of vaccines and drugs. (Companies develop vaccines and drugs against pathogens that exist and circulate in humans. Not against pathogens that do not yet exist and do not yet circulate in humans.)

Gain-of-function research of concern is performed because it is easy and fast (much faster and much easier than vaccine or drug development) and because, it is fundable and publishable. Not because it is needed.

Risk-benefit assessment and risk-mitigation review

Because gain-of function research of concern poses high--potentially existential--risks and provides limited benefits, the risk-benefit ratio for the research almost always is unfavorable and in many cases is extremely unfavorable.

Therefore, it is imperative that gain-of function research of concern be subject to national- or international-level oversight to ensure that, before the research is started, risk-benefit assessment is performed, risk-benefit profiles are acceptable, and mitigable risks are mitigated..

Effective oversight includes three components:

First, research proposals that include gain-of function research of concern must be identified

Second, a risk-benefit assessment and a risk-mitigation review must be performed. This entails enumerating anticipated risks, enumerating anticipated benefits, weighing risks and benefits, and reaching a decision either (i) to proceed as proposed, (ii) to proceed with additional risk mitigation, or (iii) not to proceed.

Third, compliance with the decision from the risk-benefit assessment and risk-mitigation review must be mandated, monitored, and enforced.

US oversight of gain-of-function research of concern

US oversight, before 2014

Before 2014, there was no national-level US oversight of gain-of-function research of concern.

US oversight, 2014-2017

In 2014-2017, there was a moratorium on HHS funding for "selected gain of function research," defined as research activities reasonably anticipated to increase transmissibility or pathogenicity of influenza, SARS, or MERS viruses. The policy was referred to as the "US Government Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses," or, for short, as the "Pause." (https://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf).

Under the Pause, 18 projects were paused.

However, at least 7 of the 18 projects that were paused were allowed to re-start almost immediately (based on a certification by the NIH Director that the projects were "urgently necessary to protect the public health or national security"). More important, other projects that met the definition for coverage under the Pause--including a project on engineering of SARS- and MERS-related coronaviruses by EcoHealth Alliance and the Wuhan Institute of Virology--were not paused, due to the failure of the NIH to identify all covered projects

US oversight, 2018-present

In 2018-present, there has been a requirement for HHS-Secretary-level risk-benefit assessment prior to awarding HHS funding for "research involving enhanced potential pandemic pathogens,"

defined as research activities reasonably anticipated to increase transmissibility or pathogenicity of a potential pandemic pathogen. The policy is referred to as the "HHS Framework for Research Involving Enhanced Potential Pandemic Pathogens," or, for short, as the "P3CO Framework" (https://www.phe.gov/s3/dualuse/documents/p3co.pdf).

Under the P3CO Framework, covered projects are to be identified by HHS funding agencies (i.e., the NIH and the CDC), and covered projects are to be forwarded to, and reviewed by, a committee appointed by the HHS Secretary (i.e., the HHS P3CO Committee).

The P3CO Framework applies to funding for proposed research and operates before funding and conduct of the research (not after completion of the research). Accordingly, identification of covered projects coverage under the policy is based on proposed research and evaluates "reasonably anticipated" results of the proposed research (not results after completion of the research). The "reasonably anticipated" standard employed by the policy is equivalent, in all respects, to the "reasonable person" standard employed in US administrative and civil law.

The definitions of the research activities covered by the P3CO Framework, and the definitions of research activities exempted from the P3CO Framework, are clear. They are as clear as in any US statute or rule having a "reasonable person" standard. The policy covers research activities reasonably anticipated to increase the transmissibility or the pathogenicity of a potential pandemic pathogen, including research activities in which neither the pathogen to be modified nor the enhanced pathogen to be generated is known to infect humans.

In principle, the P3CO Framework provides for risk-benefit assessment and risk-mitigation review for gain-of-function research of concern. *In practice, however, the P3CO Framework largely has existed only on paper.* In the four-and-one-half years since the policy was

announced, *only three projects have been reviewed*: two projects that had been carried over from the Pause, and one new project. Most covered projects--including the project on engineering of SARS- and MERS-related coronaviruses by EcoHealth Alliance and the Wuhan Institute of Virology--were not reviewed, due to a failure by the NIH to identify covered projects and to forward covered projects to the HHS P3CO Committee for review. In addition, the HHS P3CO Committee has operated with complete non-transparency and complete unaccountability. The names and agency affiliations of its members have not been disclosed, its proceedings have not been disclosed, and even its decisions have not been disclosed.

Shortcomings in US oversight of gain-of-function research of concern

US oversight of gain-of-function research of concern has grave shortcomings:

- Responsibility for oversight is assigned to federal agencies that perform research and/or fund research. This constitutes an inherent conflict of interest.
- Oversight applies only to HHS-funded research.
- Oversight is not codified in regulations with force of law, and, as a result, compliance is neither mandated, monitored, nor enforced.
- Oversight has been nullified by the failure of federal research funding agencies to identify covered projects and to forward them for review
- Oversight has been not been transparent and accountable, neither at the level of the federal research funding agencies nor at the level of the HHS P3CO Committee .

Strengthening US oversight of gain-of-function research

Rationale

Lapses in US oversight of gain-of-function research of concern may have caused the current pandemic (see Appendix 1), and could cause future pandemics The US government funded high-risk gain-of-function research and high-risk enhanced potential pathogen research at the Wuhan Institute of Virology in 2016-2019. The research overlapped the US Government Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses (the Pause) that was in effect in the 2014 to 2017, and met the criteria to be paused, but was not paused. The research also overlapped the HHS Framework for Research Involving Enhanced Potential Pandemic Pathogens (the P3CO Framework) that has been in effect in 2018 to the present, and met the criteria for federal risk-benefit review under the P3CO Framework, but did not undergo federal risk-benefit review under the P3CO Framework. The research was performed at biosafety level 2--a biosafety level that is inadequate for research with potential pandemic pathogens. The research may have generated SARS-CoV-2 or a proximal progenitor, and an accident in the research may have been responsible for entry of SARS-CoV-2 or a proximal progenitor into the human population.

These facts--and these statements indeed are facts--are an indictment of the current system of US oversight of gain-of-function research of concern and are a testament that strengthening US oversight of gain-of-function research of concern is essential.

Moving forward, any effective system of US oversight of gain-of-function research of concern must address the shortcomings of the current system:

Recommendations

- **Responsibility for US oversight of gain-of-function research of concern should be assigned to a single, independent federal agency that does not perform research and does not fund research. The oversight of research on fissionable materials by the Nuclear Regulatory Commission provides a precedent and a model.**

- **US oversight of gain-of-function research of concern should cover all US and US-funded research, irrespective of funding source, classification status, and research location.**

- **US oversight of gain-of-function research of concern should be codified in regulations with force of law and should be mandated, monitored, and enforced--in the same manner that US oversight of human-subjects research and vertebrate-animals research is codified in regulations with force of law and is mandated, monitored, and enforced.**

- **The US should call on other nations to adopt similar systems of oversight of gain-of-function research of concern.**

- **The US should call for an additional, international-level layer of oversight for the highest-risk, highest-consequence subset of gain-of-function research of concern. The oversight of research on smallpox virus by the World Health Organization Advisory Committee on Variola Virus Research provides a precedent and a model.**

Appendix 1: Origins of SARS-CoV-2

SARS-CoV-2 may have entered humans through a research-related accident.

The genome sequence of SARS-CoV-2 indicates that its progenitor was a bat coronavirus.

Bat coronaviruses are present in nature in multiple parts of China. Therefore, the first human infection could have occurred as a natural accident, with a virus passing from a bat to a human, possibly through another animal. There is clear precedent for this. The first entry of the SARS virus into the human population occurred as a natural accident in a rural part of Guangdong province in 2002.

But bat coronaviruses also are collected and studied by laboratories in multiple parts of China, including the Wuhan Institute of Virology. Therefore, the first human infection also could have occurred as a research-related accident, with a virus accidentally infecting a field-collection staffer or a laboratory staffer, followed by transmission from the staffer to the public. There also is clear precedent for this. The second, third, fourth and fifth entries of the SARS virus into human populations occurred as a laboratory accident in Singapore in 2003, a laboratory accident in Taipei in 2003, and two separate laboratory accidents in Beijing in 2004.

At this point in time, there is no scientific or other secure basis to assign relative probabilities to the natural-accident hypothesis and the research-related-accident hypothesis. Nevertheless, there are three lines of circumstantial evidence that should be noted:

First, the outbreak occurred in Wuhan, a city of 11 million persons that is more than 800 miles from, and outside the flight range of, known bat colonies with SARS-related coronaviruses.

Second, the outbreak occurred in Wuhan, on the doorstep of the laboratory that conducts the world's largest research project on bat viruses, that has the world's largest collection of bat viruses, and that possessed and worked with the bat virus that, at the time SARS-CoV-2 emerged, was the world's closest known relative of SARS-CoV-2. The laboratory actively searched for new bat viruses in bat colonies in caves in remote rural areas in Yunnan province, brought those new bat viruses to Wuhan, and then mass-produced, genetically manipulated, and studied those new bat viruses, year-round, inside Wuhan.

Third, the bat-SARS-related-coronavirus projects at the Wuhan Institute of Virology, including projects involving the construction and initial characterization of novel chimeric SARS-related coronaviruses having enhanced viral growth and enhanced lethality, used personal protective equipment (usually just gloves; sometimes not even gloves) and biosafety standards (usually just biosafety level 2) that would pose high risk of infection of field-collection or laboratory staff upon contact with a virus having the transmission properties of SARS-CoV-2.

SARS-CoV-2 may have entered humans through US-funded gain-of-function research and lapses in US oversight of gain-of-function research.

The research at the Wuhan Institute of Virology included activities that met the definition of "selected gain of function research" in the US policy in effect in 2014-2017 and that met the definition of "enhanced potential pandemic pathogen research" in the US policy in effect in 2018-present. Using US funding, provided by the NIH in 2014-2019, the Wuhan Institute of Virology: (1) constructed novel chimeric SARS-related coronaviruses that combined the spike gene of one bat SARS-related coronavirus with the rest of the genetic information of another bat SARS-related coronavirus, (2) showed that resulting viruses efficiently infected human airway

cells and efficiently replicated in human airway cells, and (3) showed that the resulting viruses exhibited up to 10,000-fold enhancement of viral growth in lungs, and up to 4-fold enhancement of lethality, in mice engineered to display human receptors on airway cells ("humanized mice").

Although this research met the definition of gain-of-function research in the US policy in effect in 2014-2017 (the Pause) and exceeded--by more three orders of magnitude--the threshold set by the NIH for enhancement of viral growth that should trigger immediate cessation of work, and although the NIH was informed of project objectives and results in annual project progress reports in 2016-2018, the NIH failed to flag the project as being covered by the policy, failed to pause the project as required by the policy, and failed to stop the project as required by the Terms and Conditions of the grant.

Although the research also met the definition of enhanced potential pandemic pathogen research in the US policy in effect in 2017-present (the P3CO Framework), and although the NIH was informed of project objectives and results in a proposal for renewal of the grant for 2019-2024, the NIH failed to identify the project as being covered by the policy, and failed to forward the proposal to the HHS Secretary for the risk-benefit assessment required by the policy.

On October 20, 2021, in response to a request from the Ranking Member of the House Oversight Subcommittee, the NIH Acting Director, Lawrence A. Tabak, D.D.S., Ph.D., released a letter on NIH-funded research on bat SARS-related coronaviruses conducted at the Wuhan Institute of Virology and Wuhan University in 2014-2019 (https://www.documentcloud.org/documents/21674679-tabak-letter-to-comer-oct-20-2021). The Tabak letter addressed: (1) NIH funding under grant AI110964, awarded by the NIH to EcoHealth Alliance with subcontracts to the Wuhan Institute of Virology and Wuhan University;

(2) the virus WIV1 SHC014 S (mis-rendered as "SHC014 WIV1"), a virus constructed and characterized in Wuhan using NIH funding under NIH grant AI110964;; and (3) the possibility that the virus WIV1 SHC014 S was a proximal progenitor of SARS-CoV-2.

WIV1 SHC014 S is a novel chimeric SARS-related coronavirus that combines the spike gene of one bat SARS-related coronavirus with the rest of the genetic information of another bat SARS-related coronavirus. It is an artificial, laboratory-constructed virus that has no counterpart in viruses that circulate in nature. It is one of at least three artificial, laboratory-constructed chimeric coronaviruses that were constructed by EcoHealth Alliance and its Wuhan partners using NIH funding and that were shown to infect human airway cells, to replicate in human airway cells, and to exhibit 10,000-fold higher viral growth and higher lethality than the parental natural coronavirus in infection studies in mice engineered to display human receptors on airway cells ("humanized mice"; https://theintercept.com/document/2021/09/08/understanding-the-risk-of-bat-coronavirus-emergence/; https://republicans-oversight.house.gov/wp-content/uploads/2021/10/Year-5-EHAv.pdf).

The year-4 progress report for the first 5-year term of the NIH grant (submitted to the NIH in March 2018) and the proposal for the second term 5-year term of the NIH grant (submitted to the NIH in November 2018) reported the construction of the three chimeras, the 10,000-fold enhanced viral growth in humanized mice of the three chimera, and the enhanced pathogenicity in humanized mice of one of the three chimeras (https://theintercept.com/document/2021/09/08/understanding-the-risk-of-bat-coronavirus-emergence/).

The year-5 proposal for the first 5-year term of the NIH grant (submitted to NIH in August 2021, more than two years overdue, and released to the Ranking Member of the House Oversight Subcommittee together with the Tabak letter) reported that the chimeras exhibited enhanced viral growth in brains as well as in lungs of humanized mice, and exhibited 2- to 4-fold increased lethality in humanized mice (https://republicans-oversight.house.gov/wp-content/uploads/2021/10/Year-5-EHAv.pdf).

The Terms and Conditions of the first 5-year NIH grant stated (https://theintercept.com/document/2021/09/08/understanding-the-risk-of-bat-coronavirus-emergence/):

> Per the letter dated July 7, 2016 to Mr. Aleksei Chmura at EcoHealth Alliance, should any of the MERS-like or SARS-like chimeras generated under this grant show evidence of enhanced virus growth greater than 1 log over the parental backbone strain you must stop all experiments with these viruses and provide the NIAID Program Officer and Grants Management Specialist, and Wuhan Institute of Virology Institutional Biosafety Committee with the relevant data and information related to these unanticipated outcomes.

The term "1 log" means "a factor of 10". EcoHealth Alliance and its Wuhan partners created novel chimeras of SARS-related coronaviruses that showed enhanced viral growth by greater than a factor of 10,000...which exceeded, *by three orders of magnitude*, the trigger point for stopping work and reporting results to NIH under the Terms and Conditions of the NIH grant.

The Tabak letter confirms that research reported in the reported in the year-4 and year-5 progress reports of the first 5-year grant and in the renewal proposal for the second 5-year grant--research

in Wuhan that generated a potential pandemic pathogen with a greater than 10,000-fold enhanced viral growth, enhanced pathogenicity, and enhanced lethality in humanized mice-- occurred. The Tabak letter thus confirms that NIH funds supported gain-of-function research of concern and construction and characterization of an enhanced potential pandemic pathogen--a pathogen reasonably anticipated, indeed likely, to have enhanced transmissibility and/or pathogenicity in humans--in Wuhan.

The Tabak letter reveals that EcoHealth Alliance and it Wuhan partner failed to report to NIH in a timely manner that they had obtained evidence of enhanced viral growth greater than 1 log over the parental backbone strain. Thus the Tabak letter confirms that EcoHealth Alliance and its Wuhan partner violated the Terms and Conditions of the first 5-year grant,

The Tabak letter also reveals that EcoHealth Alliance failed to submit the year-5 progress report for the first 5-year grant report until more than two years after the submission deadline. Thus the Tabak letter also confirms that EcoHealth Alliance and its Wuhan partner again violated the Terms and Conditions of the first 5-year grant,

The Tabak letter correctly states that WIV1 SHC014 S and the other novel chimeric SARS-related viruses reported to the NIH by EcoHealth Alliance and its Wuhan partners in their 2018 grant progress report and 2018 grant renewal proposal are insufficiently closely related to SARS-CoV-2 to have served as a proximal progenitor of SARS-CoV-2.

However, the Tabak letter leaves unstated the crucial fact that the NIH has received no information on novel chimeric SARS-related viruses constructed by EcoHealth Alliance and its Wuhan partners subsequent to the 2018 grant progress report and 2018 grant renewal proposal., and therefore that the NIH cannot rule out the possibility that the project created a proximal

progenitor of SARS-CoV-2, and cannot even rule out the possibility that the project used NIH funding to create a proximal progenitor of SARS-CoV-2.

The Tabak letter also leaves unanswered the questions of why the NIH, which was provided with relevant data in March of 2018 and again in November of 2018, and which became aware of the failure to submit the year-5 progress report in 2019: (1) failed to act on the violations of the Terms and Conditions of the first 5-year grant, (2) awarded a second 5-year grant period despite the violations of the Terms and Conditions of the first 5-year grant, (3) awarded a second 5-year grant period for a project that proposed continuation of enhanced potential pandemic pathogen research--specifically proposing to construct and characterize additional novel chimeric SARS-related coronaviruses--without forwarding the proposal for HHS-level risk-benefit review as required under the HHS P3CO Framework, and (4) falsely asserted that NIH funding had not supported gain-of-function research or enhanced potential pandemic pathogen research in Wuhan.

Appendix 2

Policy document: US Government Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses (https://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf).

Policy document: HHS Framework for Research Involving Enhanced Potential Pandemic Pathogens (https://www.phe.gov/s3/dualuse/documents/p3co.pdf).

U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses

October 17, 2014

U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses

Gain-of-function studies, or research that improves the ability of a pathogen to cause disease, help define the fundamental nature of human-pathogen interactions, thereby enabling assessment of the pandemic potential of emerging infectious agents, informing public health and preparedness efforts, and furthering medical countermeasure development. Gain-of-function studies may entail biosafety and biosecurity risks; therefore, the risks and benefits of gain-of-function research must be evaluated, both in the context of recent U.S. biosafety incidents and to keep pace with new technological developments, in order to determine which types of studies should go forward and under what conditions.

In light of recent concerns regarding biosafety and biosecurity, effective immediately, the U.S. Government (USG) will pause new USG funding for gain-of-function research on influenza, MERS or SARS viruses, as defined below. This research funding pause will be effective until a robust and broad deliberative process is completed that results in the adoption of a new USG gain-of-function research policy[1]. Restrictions on new funding will apply as follows:

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. The research funding pause would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity.*

In parallel, we will encourage the currently-funded USG and non-USG funded research community to join in adopting a voluntary pause on research that meets the stated definition.

The deliberative process that will ensue during the period of the research pause will explicitly evaluate the risks and potential benefits of gain-of-function research with potential pandemic pathogens. The presumptive benefits that are generally identified in pursuing this type of research are stated in terms of enhanced ability for earlier awareness of naturally emerging dangerous pandemic pathogens or in the development of medical products in anticipation of such emergence.

However the relative merits of gain-of-function experimental approaches must be compared ultimately to potentially safer approaches. The deliberative process will offer recommendations for risk mitigation, potential courses of action in light of this assessment, and propose methodologies for the objective and rigorous assessment of risks and potential benefits that might be applied to the approval and conduct of individual experiments or classes of experiments. Although the gain-of-function studies that fall within the scope of research subject to the funding pause will be a starting point for deliberations, the suitability of other types of gain-of-function studies will be discussed. It is feasible that the discussion could lead to suggestions of broadening the funding pause to include research with additional pathogens,

[1] An exception from the research pause may be obtained if the head of the USG funding agency determines that the research is urgently necessary to protect the public health or national security.

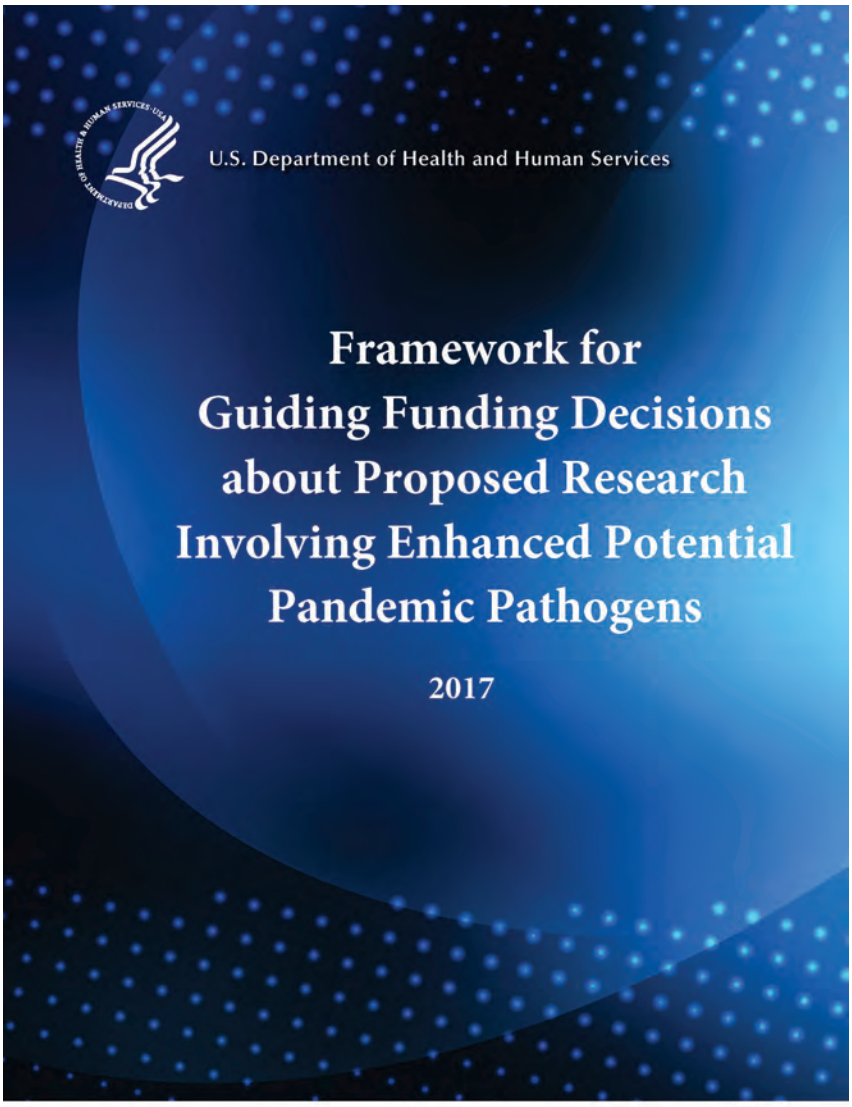
however, federal Departments and Agencies who fund, support, or perform research should be consulted prior to any additional pathogens being added to the scope of the funding pause.

The deliberative process is envisioned to be time-limited, to involve two distinct, but collaborating, entities, and to be structured to enable robust engagement with the life sciences community. As a first step, the National Science Advisory Board for Biosecurity (NSABB) will be asked to conduct the deliberative process described above and to draft a set of resulting recommendations for gain-of-function research that will be reviewed by the broader life sciences community. The NSABB will serve as the official federal advisory body for providing advice on oversight of this area of dual use research, in keeping with federal rules and regulations.

As a second step, coincident with NSABB recommendations, the National Research Council (NRC) of the National Academies then will be asked to convene a scientific conference focused on the issues associated with gain-of-function research and will include the review and discussion of the NSABB draft recommendations. This NRC conference will provide a mechanism both to engage the life sciences community as well as solicit feedback on optimal approaches to ensure effective federal oversight of gain-of-function research. The life sciences community will be encouraged to provide input through both the NRC and NSABB deliberative processes.

The NSABB, informed by NRC feedback, will deliver recommendations to the Secretary of Health and Human Services, the Director of the National Institutes of Health, and the heads of all federal entities that conduct, support, or have an interest in life sciences research (including the Assistants to the President for Homeland Security and Counterterrorism and for Science and Technology). The final NSABB recommendations and the outcomes of the NRC conference will inform the development and adoption of a new U.S. Government policy governing the funding and conduct of gain-of-function research. Upon adoption of a federal gain-of-function policy, the U.S. Government will declare the end of the research funding pause.

The life sciences community will be informed of progress at regular intervals. The estimated time-line is six months for completion of the two deliberative steps (culminating in delivery of the NSABB recommendations to the HHS Secretary) and three months for the development, approval, and publication of the policy, with the goal of completing the entire process in less than one year from declaration of the research funding pause.


U.S. Department of Health and Human Services
Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens
2017

Department of Health and Human Services Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens

Section I. Purpose and Principles

Research involving potential pandemic pathogens (PPPs) is essential to protecting global health and security. However, there are biosafety and biosecurity risks associated with undertaking such research that must be adequately considered and appropriately mitigated in order to help safely realize the potential benefits. The *HHS Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens* (*HHS P3CO Framework*) is intended to guide HHS funding decisions on individual proposed research that is reasonably anticipated to create, transfer, or use enhanced PPPs. This *HHS P3CO Framework* is responsive to and in accordance with the *Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight* issued by OSTP on January 9, 2017[1] and supersedes the previous *Framework for Guiding Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets*[2]. The *HHS P3CO Framework* ensures a multidisciplinary, department-level pre-funding review and evaluation of proposed research meeting the scope outlined herein to help inform funding agency decisions. In so doing, the *HHS P3CO Framework* seeks to preserve the benefits of life sciences research involving enhanced PPPs while minimizing potential biosafety and biosecurity risks.

Section II. Scope and Definitions

For the purposes of this *HHS P3CO Framework*:

A. A ***potential pandemic pathogen*** **(PPP)** is a pathogen that satisfies **both** of the following:

   1. It is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations; and

   2. It is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.

B. An **enhanced PPP** is defined as a PPP resulting from the enhancement of the transmissibility and/or virulence of a pathogen. Enhanced PPPs do not include naturally occurring pathogens that are circulating in or have been recovered from nature, regardless of their pandemic potential.

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[1] Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight. U.S. Government, January 2017.

[2] Framework for Guiding Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets. U.S. Government, February 2013.

C. To the extent that transmissibility and/or virulence of PPPs are modified in the following categories of studies, the resulting pathogens are not considered to be enhanced PPPs for the purposes of this Framework[3]:

   1. Surveillance activities, including sampling and sequencing; and
   2. Activities associated with developing and producing vaccines, such as generation of high growth strains.

D. Proposed intramural and extramural life sciences research that is being considered for funding and that has been determined by the funding agency as reasonably anticipated to create, transfer, or use enhanced PPPs is subject to additional HHS department-level review as outlined herein.

E. A pathogen previously considered by an agency to be an enhanced PPP should no longer be so considered if the HHS and the White House Office of Science and Technology Policy, in consultation with the Departments of Defense, Homeland Security, Agriculture, and Justice, generally acting through the Federal Bureau of Investigation, jointly determine, on the basis of additional information that has been developed about the risks or the benefits of that pathogen's creation, transfer, or use, that the department-level review processes outlined in this framework are no longer appropriate.

[3] For additional guidance and examples of activities that would and would not be considered to involve enhanced PPP see *Recommendations for the Evaluation and Oversight of Proposed Gain-of-Function Research*. National Science Advisory Board for Biosecurity, May 2016.

Box 1. Criteria for guiding HHS funding decisions on proposed research that involves, or is reasonably anticipated to involve, creation, transfer, or use of enhanced PPPs.

Department-level review of proposed research reasonably anticipated to create, transfer, or use enhanced PPPs will be based on the following criteria:

1) The research has been evaluated by an independent expert review process (whether internal or external) and has been determined to be scientifically sound;
2) The pathogen that is anticipated to be created, transferred, or used by the research must be reasonably judged to be a credible source of a potential future human pandemic;
3) An assessment of the overall potential risks and benefits associated with the research determines that the potential risks as compared to the potential benefits to society are justified;
4) There are no feasible, equally efficacious alternative methods to address the same question in a manner that poses less risk than does the proposed approach;
5) The investigator and the institution where the research would be carried out have the demonstrated capacity and commitment to conduct it safely and securely, and have the ability to respond rapidly, mitigate potential risks and take corrective actions in response to laboratory accidents, lapses in protocol and procedures, and potential security breaches;
6) The research's results are anticipated to be responsibly communicated, in compliance with applicable laws, regulations, and policies, and any terms and conditions of funding, in order to realize their potential benefit;
7) The research will be supported through funding mechanisms that allow for appropriate management of risks and ongoing Federal and institutional oversight of all aspects of the research throughout the course of the research; and
8) The research is ethically justifiable. Non-maleficence, beneficence, justice, respect for persons, scientific freedom, and responsible stewardship are among the ethical values that should be considered by a multidisciplinary review process in making decisions about whether to fund research involving PPPs.

Section III. Review and Oversight Framework

A. The identification, review, and oversight of research subject to department-level review will require responsibilities (Figure 1) of the:

- Funding agency considering funding the proposed research; and
- HHS.

Figure 1: Overview of Responsibilities under the HHS P3CO Framework

Entity	Responsibilities
Funding agency	• Conduct standard scientific merit review; • Refer proposed research that is reasonably anticipated to create, transfer, or use enhanced PPPs for departmental-level review; • Provide relevant information necessary for departmental-level review; • Participate in departmental-level review process, as requested; • Consider the recommendations resulting from the departmental-level review; • Make a funding decision, stipulating terms and conditions of award including additional risk mitigation measures if appropriate; • Report relevant information on funding decisions to HHS and OSTP; • Ensure implementation of and adherence to required risk mitigation procedures and other terms/conditions of award, if funded.
HHS	• Convene a multidisciplinary group to review proposed research that has been determined by the funding agency as being reasonably anticipated to create, transfer, or use enhanced PPPs; • Critically evaluate the proposed research including the risk/benefit assessment and proposed risk mitigation plan; • Consider the eight criteria for guiding HHS funding decisions (Box 1) and additional relevant factors and information; • Develop recommendations on acceptability for HHS funding, including suggestions for additional risk mitigation measures and/or terms and conditions of award, if funded.

B. The HHS department-level review will evaluate proposed research referred by the funding agency that meets the scope outlined in Section II. This review and evaluation will be guided by the criteria listed in Box 1. The evaluation will include consideration of a:

- Risk/benefit analysis of the proposed research;
- Risk mitigation plan; and
- Additional relevant factors.

C. A department-level review will result in recommendations to the funding agency on whether the proposed research is acceptable for HHS funding and what, if any, additional risk mitigation measures should be incorporated into the terms and conditions of award, if funded.

D. If funded, research that is reasonably anticipated to create, transfer, or use an enhanced PPP may require additional risk mitigation strategies which may include, but are not limited to:

- Modification of the design or conduct of the research;
- Application of specific or enhanced biosecurity or biosafety and biocontainment measures;

- Evaluation of existing evidence of medical countermeasures (MCM) efficacy, or experiments conducted to determine MCM efficacy against agents or toxins resulting from the research; and
- Methodologies for responsible communication of results.

Section IV. HHS Department-level Review

A. Proposed research that is being considered for funding by the HHS funding agency, is deemed to be scientifically meritorious by an independent internal or external review process, and has been determined by the funding agency to be reasonably anticipated to create, transfer, or use enhanced PPPs must be referred for HHS department-level review.

B. The purpose of the department-level review is to provide a multidisciplinary, pre-funding review and evaluation of proposed research that meets the scope outlined in Section II to recommend whether HHS funding is appropriate, and if so, to help identify the appropriate risk mitigation strategies. The following disciplines should be represented during the department-level review: scientific research, biosafety, biosecurity, MCM development and availability, law, ethics, public health preparedness and response, biodefense, select agent regulations, and public health policy, as well as the funding agency perspectives and other relevant areas. The HHS department-level review group may include non-voting *ex officio* and/or *ad hoc* members from HHS and other federal departments and agencies as deemed appropriate by the Review Group Chair.

C. Extra care in the department-level review should be given to proposed research that is reasonably anticipated to:

- Enhance the harmful consequences of the pathogen;
- Disrupt immunity or the effectiveness of an immunization against the pathogen without clinical or agricultural justification;
- Confer to the pathogen resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that pathogen or facilitate the pathogen's ability to evade detection methodologies;
- Increase the stability, transmissibility, or the ability to disseminate the pathogen;
- Alter the host range or tropism of the pathogen;
- Enhance the susceptibility of a host population to the pathogen; or
- Generate or reconstitute an eradicated or extinct pathogen.

D. The HHS department-level review may result in the following recommendations:

- Research is acceptable for HHS funding;
- Research is not acceptable for HHS funding;
- Research is acceptable for HHS funding on the condition that certain experiments are modified;

- Research is acceptable for HHS funding on the condition that certain risk mitigation measures are employed at the federal and/or institutional level; or
- Other recommendations, as deemed appropriate.

For research determined to be not in accordance with all of the criteria for guiding HHS funding decisions on proposed research reasonably anticipated to create, transfer, or use enhanced PPPs, a recommendation will be that the research is not acceptable for HHS funding.

Section V. Evaluation of the HHS P3CO Review Process

HHS will periodically re-evaluate and modify this review process, as necessary, to reflect scientific advances and changes to the regulatory landscape. To help inform such evaluations, and to enhance transparency and public engagement in the review and oversight process for enhanced PPP research, HHS will periodically ask the National Science Advisory Board for Biosecurity to review the process described herein.

Written remarks to accompany the testimony of Steven Quay, MD, PhD

"Revisiting Gain of Function Research: What the Pandemic Taught Us and Where Do We Go From Here"
Senate Committee on Homeland Security and Governmental Affairs'
Subcommittee on Emerging Threats and Spending Oversight

Subcommittee Chair Senator Margaret Wood Hassan, Ranking Member Senator Rand Paul, Members of the Governmental Affairs' Subcommittee on Emerging Threats and Spending Oversight, invited Senator committee participants, Ladies and Gentlemen.

I am honored to participate with my esteemed colleagues, Drs. Ebright and Esvelt, in this forum entitled: "Revisiting Gain of Function Research: What the Pandemic Taught Us and Where Do We Go From Here."

My prepared remarks will take about seven minutes. I will cover six topics:

- I will begin with an overview of the evidence related to the origin of the pandemic. My conclusion from two years of investigation is that the pandemic began with a laboratory-acquired infection.
- The virus has three genomic regions that have the signature of synthetic biology, that is, gain-of-function research.
- Two of those regions involve the three types of academic gain-of-function research that are permitted.
- One region has features of the two types of forbidden gain-of-function research that are associated with bioweapons development, asymptomatic transmission and immune system evasion.
- Finally, I will present evidence of synthetic biology research at the Wuhan Institute of Virology being conducted in low level, BSL 2/3 facilities, in December 2019 on the Nipah virus, which is >60% lethal but is not naturally airborne. This is the most dangerous research I have ever encountered.
- I will close with five recommendations for future gain-of-function research.

Where did the pandemic begin?

The competing hypotheses are a natural spillover at the Huanan Seafood market in Wuhan China and a laboratory-acquired infection, most likely at the Wuhan Institute of Virology or WIV. Before December 2019, the WIV had published over 65% of all coronavirus scientific papers in the world. Until it was removed from the WIV website at 2 am local time, September 19, 2019, they maintained a database of over 21,000 viruses collected over two decades, in part with NIH funding. To my knowledge, no western scientist or organization has had access to this database since the pandemic began.

Two papers published last week by western scientists and a flurry of coordinated news coverage purported to end the debate, stating the pandemic began in the market in December 2019 and even claiming the market contained infected animals. There are at least six problems with these papers:

- No animal has ever been found to be infected with CoV-2. Hundreds from the market were tested and over 80,000 throughout China were all negative.

- All environmental specimens from the market were the result of human infection, not animal infection. For comparison, with the 2003 SARS infection >90% of market animals were infected.
- These papers suppress cases from the eastern side on the Yangtze River, near the WIV, for no apparent reason. These include cases identified during the World Health Organization investigation.
- Scientists agree that the most ancestral version of CoV-2 that infected humans, named Lineage A, did not infect any patient from the market.
- Orthogonal methods agree that the virus was circulating in Wuhan in the fall of 2019 and well before the market cases. I, as well as many others, believe the market cases were a super-spreader event.

My own research has identified the earliest cluster of hospitalized patients with both the Lineage A and Lineage B virus at the People's Liberation Army Hospital in Wuhan, identified on the chart shown here. This hospital is about 3 km from the WIV and along Line 2 of the Wuhan subway system. My research also showed that all early cases were along this same subway line, one of nine in Wuhan, and that the probability this was by chance is one in 68,000. The Line 2 COVID Conduit, as I called it, includes the PLA Hospital, the WIV, the market, and at the last stop, the international airport. You can literally walk down into the subway system and next exit in the world in London, Paris, Dubai, and New York City, all before having any symptoms. In the fall of 2019 one million people a day used Line 2 and modelling by others suggested the pandemic could not have occurred without the spreading impact of Line 2.

What are the gain-of-function features of SARS-CoV-2?

First, gain-of-function research is defined as making artificial changes to a microbe in a laboratory, seeing what new properties it acquires by those changes, and then often performing additional research to find vaccines or therapeutic that can stop this synthetic virus.

So create something that doesn't exist in nature and see if you can kill it.

The three kinds of gain-of-function research that have been agreed are acceptable in academic work are changing trophism, that is changing the host animal; changing infectivity (ease of transmission) and/or pathogenicity (how dangerous it is). Two kinds of gain-of function research have been agreed by scientists as off limits, as they have bioweapons features. These are making infections hard to detect and making viruses that can evade the immune system.

SARS-CoV-2 has features of all five kinds of gain-of-function research, including the off-limits work.

Host selection, infectivity, and pathogenicity in SARS-CoV-2 are governed by the two-step verification system used by coronaviruses to infect cells. Step one is the handshake between the receptor binding domain and the human ACE2 receptor on the surface of the human respiratory system. Step two is the spike protein cleavage site, in CoV-2 the so-called furin cleavage site, which puts a cut in the spike protein. At that point the virus injects its genetic material into the cell and begins the 12 hour or so process of replicating. This ends with the cell dissolving and thousands of new viruses being released.

In SARS-CoV-2, the receptor binding domain was largely perfected for human infection. Specifically, the first virus to infect humans had only 17 mutations it could make out of 4000 that would improve the ACE2 handshake. With SARS1, the first human infections were with a virus that had only 15% of the mutations needed for the epidemic.

How and where did the virus learn to infect humans?

In the SARS1 epidemic, once an antibody test was available to identify people who had been infected, stored blood samples were tested for previous, undiagnosed infections. Workers in the market had a 20% positivity while in the general population you could find about 1% of people who were infected. These people were the training ground on which SARS1 learned to infect humans and learn to support human-to-human spread.

Did we find a similar training ground in stored human specimens from Wuhan before the pandemic?

No. 36,000 blood specimens were tested, and none were positive. If SARS2 was like SARS1 we would have expected at least 360 positive stored specimens.

Where could SARS2 have learned to infect human?

Replicating a virus in human cell cultures, that is, a test tube would do it. So would passing the virus in mice genetically modified to contain human lung tissue, so-called humanized mice. And we know that a US coronavirus researcher, funded by the NIH, provided the WIV with his laboratory's humanized mice for doing this type of research.

The other SARS2 feature that contributed to infectivity and pathogenicity was the furin cleavage site. Many viruses use a host cell enzyme or scissors to cut a virus cell surface attachment protein as the last step before infectivity. And designing a virus in the laboratory that uses the enzyme furin by putting a synthetic furin cleavage site is a common go-to gain-of-function exercise. In fact, since 1992, at least 14 publications have described adding a furin cleavage site to a virus that didn't have one, including a study from the WIV. 14 out of 14 times it makes the viruses nastier.

In fact, a grant application from 2018 involving a collaboration between US and Chinese scientists proposed synthetically inserting a "human specific furin cleavage site" into a bat virus. Finding the exact furin cleavage site in SARS2 that is also found naturally in an important human lung protein, ENAC, that controls water flow into the lung, is very suspicious. And the backbone of SARS2, about 96% of the virus, is identical to several bat viruses. Interestingly, SARS2 is so adapted to the human host that it can no longer infect bat cells in culture.

The SARS2 furin cleavage site is also unusual in two ways. In the 1000 years since the SARS2-related viruses separated from the other related beta coronaviruses, there has never been a virus with a furin cleavage site. It is also unusual in that it uses a rare genetic sequence that has also never been used by these related viruses in nature. The sequence is a common genetic sequence used in the lab when gene jocks juice up viruses.

The furin cleavage site in SARS2 also explains why this virus but not SARS1 can infect the brain, heart, lungs, kidneys, and other organs. This is because these organs also have the furin enzyme on their surface.

What about gain-of-function features related to asymptomatic spread and immune system evasion?

Since these two features would greatly increase the pandemic potential of a virus, governments and academic scientists have agreed not to conduct gain-of-function research in these areas.

Where does SARS2 come in?

SARS2 contains a protein called ORF8, so named because it is the eighth protein in the SARS2 genome. It is one of the only proteins that is not part of the finished virus or is involved in taking over the cellular machinery that makes new viruses. ORF8 is diabolical. It is made early after an infection before other viral proteins begin to be synthesized. At this point the cell is largely unaware it is infected and hasn't mounted any defenses. ORF8 enters the blood stream and interacts with the immune system, doing two things.

First, it blocks the production of interferon. Interferon has two important functions:

First, it is a blunt weapon against infections that is used early by the body to slow down an infection, allowing time for antibodies to be produced and T-cells to respond. And second, it produces the familiar symptoms of an infection: fever, chills, sweating, red skin. The symptoms of an infection are not directly from the microbe itself but are from the body's response to the presence of a microbe. Take interferon away and you have asymptomatic spread. I am aware of no other new respiratory virus that is asymptomatic when it first entered the human population. If you remember back to the early days of COVID, no one thought we could be missing infections because of lack of symptoms. We now know that 40% of COVID from the beginning was asymptomatic.

The other property of ORF8 is that it interferes with the immune system's process of making antibodies and teaching T-cells about the virus. This so-called MHC antigen presentation system is important for fighting infections. The AIDS virus is the poster child of viruses that become chronic infections because, among other things, it inhibits the normal immune system response. No one knew about ORF8 and these features when the vaccine target was being selected and so immunity from vaccination does not include inhibiting ORF8. Interestingly, in a natural infection your body recognizes ORF8 as a highly foreign protein and actually makes more antibodies against it than any other protein.

What does this have to do with gain-of-function research at the WIV?

Prior to 2019, the WIV had conducted extensive research on optimizing the ORF8 gene and its function and on creating a synthetic biology pathway for manipulating this protein and putting it in viruses in the laboratory. This work was found in two master theses from students at the WIV that were never translated from Mandarin nor did they ever lead to publications. They were in fact found online by a group of amateur investigators self-named DRASTIC. I have found no western virologist that was doing research on ORF8 before the pandemic.

Knowing from the beginning that SARS2 had these three genetic features, that is, an optimized receptor binding domain, the effects of the furin cleavage site on transmissibility and multi-organ affinity, and the properties of ORF8 would have significantly helped in reducing the pandemics impact for three reasons:

- Human-to-human spread was accruing from the beginning and did not have to be acquired slowly, like with SARS1. The world lost almost a month of response time while public health officials made pronouncements about lack of human-to-human spread;
- Rapid spread within the body because of the humanized furin cleavage site, beginning in the lungs but leading often to multi-organ attack, could have guided treatment to better outcomes;
- And finally, knowing that 40% of cases were asymptomatic and that vaccines might be improved by including immunizing against ORF8 could have been easily done and might have improved vaccine efficacy, as well as not missing early asymptomatic cases.

Has gain-of-function research been useful to the COVID19 response or other public health infectious disease emergencies?

In looking at the collected gain-of-function research over approximately two decades, I have found no findings that could reasonably be considered to have helped in either the COVID pandemic or other smaller epidemics. At this point we know that an mRNA vaccine can be designed within literally days of a new outbreak once the pathogen is sequenced, and large-scale manufacturing can begin soon thereafter. This capability has now been fully road tested and provides, in my opinion, the best defensive capability against future microbes.

It is also important to point out that gain-of-function research is a tiny sliver of all the research funded by NIH. Specifically, there were over 36,000 RO1 grants funded by NIH in 2020, the latest year with statistics. Of these, the self-described "gain-of-function on potential pathogens" research grants numbered only twenty-one in the latest funding year. Even expanding this by tenfold with a less stringent definition of gain-of-function would mean we are talking about less than 1% of all NIH research funding.

I cannot imagine a scenario where, but for this tiny research effort, a new pandemic occurs.

What reforms should be considered in order to assure that such research is conducted in a safe and transparent manner?

While I find no actual benefit of gain-of-function research, I believe efforts to ban it, given the vested interests of literally the entire virology community, and maybe others, is a hill too steep to climb. A proposal that I believe is achievable is the placement of all select agent research within the existing institutional review board structure used for human clinical trials. The requirement to explain the cost-benefit of a particular set of experiments to unaffiliated lay people and community members could place guardrails on the field and eliminate the most dangerous research. The argument that the research is too complex to explain to non-scientists fails when you point out that chimeric antigen receptor genetic engineering of the human immune system to fight cancer is routinely placed within the oversight of the IRB system. This research is arguably more complex than gain-of-function work. In addition, the IRB system is an international standard that is used everywhere, including in China.

My second reform is to separate the governmental oversight of this research from the funding agency. We have now documented the failure of the internal NIH system, the so-called P3CO, to provide adequate oversight. The model is atomic energy research, which is funded by the Department of Defense, but which is overseen by the Atomic Energy Commission.

My third suggestion is to place the western technology of biotechnology under export controls and monitoring. There are ways to build into these systems a forensic and law enforcement capability that could, for example, with probably cause and a court-issued warrant allow

What happens if we have these hearings, and nothing changes?

A significant part of my prepared statement is derived from the evidence that I and others have collected related to the pandemic that can be sourced from outside of the WIV. In a court of law much of this would be called circumstantial evidence. While sometimes circumstantial evidence is characterized as of inferior quality it certainly does not need to be. Murders can be solved when the weapon or even the victim cannot be found. Convictions based solely on such circumstantial evidence happen all the time. But my final statement is based on actual evidence from inside the WIV. Please show the next chart.

In December 2019 five bronchial lavage specimens were taken from patients in Wuhan and sent to the WIV for analysis. The patients had pneumonia and using a sequencing machine from a US company was used to identify SARS2. The paper that was written about these patients was quickly published the first week of February and this paper has been viewed millions of times. The WIV also published the raw data that came from the specimen as well. These samples were massively expanded, using a PCR like process, and ultimately yielded tens of millions of reads of genetic material.

We took these specimen reads and conducted a forensic analysis, making three observations:

- First, we confirmed they contained the SARS2 virus.
- Second, we identified 20 unexpected contaminants in the specimens that we suspected to be the inadvertent amplification of other research going on in the laboratory. Things not expected to be found in a human specimen like honey suckle genes or a horse virus. For 19 of the 20 unexpected contaminants, we then found published research from the previous two years, confirming that the lab had indeed been working on these unexpected genes. This also validated that our methods could detect covert research efforts.
- One contaminant was not accounted for in published papers. The chart shows this finding: a portion of the Nipah virus genome in a laboratory vector commonly used for synthetic biology.

The Nipah virus is a smaller virus than SARS2 and is much less transmissible. But it is one of the deadliest viruses, with a >60% lethality. This is 60-times deadlier than SARS2. The lab where the human specimens were processed is not the highest level biosafety lab, BSL-4, but was in the BSL-2 or -3 facility.

Why were they conducting synthetic biology research in December 2019 on the Nipah virus? I cannot speculate.

But a laboratory-acquired infection with a modified Nipah virus would make the COVID19 pandemic look like a walk in the park.

The work of this committee is critical to protecting the American people and, really, the world at large, from future manmade pandemics. Thank you for the opportunity to speak before this committee.

Senate Homeland Security and Governmental Affairs Committee
Subcommittee on Emerging Threats and Spending Oversight

Credible pandemic virus identification will trigger the immediate proliferation of agents as lethal as nuclear devices

Testimony of Professor Kevin M. Esvelt, Massachusetts Institute of Technology

Introduction

Senator Hassan, Senator Paul, and members of the subcommittee, thank you for inviting me to testify on the subject of emerging threats from pandemic virus identification and enhancement research.

A million Americans have lost their lives to COVID-19, more than have perished in combat in all of our nation's foreign wars. **A pandemic virus can demonstrably kill more people than any single operational nuclear weapon.**[1]

For 75 years, the United States has successfully kept nuclear capabilities out of the hands of terrorists. Due to recent technological advances that have made it easy to assemble viruses from synthetic DNA, pandemics now represent a considerably greater challenge for nonproliferation, not least because they are wrongly viewed as a problem for health agencies that largely lack security expertise.

The threat of pandemic proliferation is still nascent: we do not yet know of any credible examples of novel viruses likely to cause a new pandemic if released.

If numerous pandemic-capable viruses are credibly identified and their genome sequences are shared with the world – as is the goal of well-meaning programs operated by the U.S. National Institutes of Health and the U.S. Agency for International Development – individual terrorists will gain the ability to unleash more pandemics at once than would naturally occur in a century.[2]

We know of at least one historical individual who both sought to acquire weapons of mass destruction for use against civilians and possessed an educational background, technical skills, and resources that would have allowed him to assemble and release viruses had he lived today.[3] Other highly capable mass murderers, including the Unabomber and certain technically skilled gunmen, would plausibly have sought out the ability to cause pandemics had they been given the opportunity. Still others may have remained unknown to us because they lacked the capability to cause sufficient damage and consequently declined to act. These examples suggest that at least one such would-be terrorist may be active today.

As a practicing biotechnologist who specializes in harnessing evolution using viruses as tools and inventing methods of editing laboratory organisms that will controllably spread in the wild,[4] **I am reasonably confident that pandemic virus identification represents a greater near-term threat to national security than anything else in the life sciences – and a more severe proliferation threat than nuclear has ever posed.**

To help understand the framework for this conclusion, my assessment considers questions of threat magnitude, proliferation, credibility, and utility. I conclude by outlining congressional actions that can delay the identification of pandemic-capable viruses long enough for us to build adequate defenses using new technologies.

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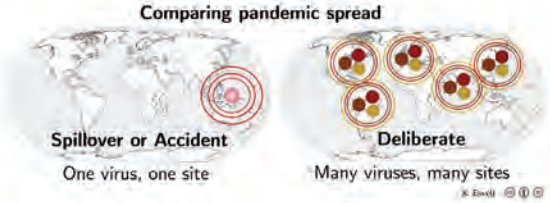
[1] Adam, "15 Million People Have Died in the Pandemic, WHO Says"; "NUKEMAP by Alex Wellerstein."
[2] Willman and Muller, "A Science in the Shadows"; Rogin, "The U.S. Government Is Rushing to Resume Risky Virus Research. Not so Fast"; Grange et al., "Ranking the Risk of Animal-to-Human Spillover for Newly Discovered Viruses."
[3] Danzig et al., "Aum Shinrikyo: Insights into How Terrorists Develop Biological and Chemical Weapons."
[4] Esvelt, Carlson, and Liu, "A System for the Continuous Directed Evolution of Biomolecules"; DeBenedictis et al., "Systematic Molecular Evolution Enables Robust Biomolecule Discovery"; Esvelt et al., "Concerning RNA-guided Gene Drives for the Alteration of Wild Populations"; Noble et al., "Daisy-Chain Gene Drives for the Alteration of Local Populations."

Deliberate pandemics can inflict more harm than any nuclear device or natural pandemic

SARS-CoV-2 has demonstrated that a pandemic virus spreading from a single point of origin can cause more deaths than any operational nuclear warhead, inflicting trillions in economic damages and disrupting lives worldwide. A single point of origin is the expected outcome of both natural spillovers and of lab-associated accidents, one of which was the cause of the Covid-19 pandemic in 2019.[5] At this time, the available evidence appears insufficient to determine which was responsible.

However, we can safely conclude that a deliberate pandemic involving the same virus would have been worse. A malevolent actor could have released SARS-CoV-2 in multiple travel hubs, resulting in considerably faster spread across the world and many more infections and deaths before the advent of vaccines. Indeed, unless a proven vaccine is already stockpiled in large numbers near major cities, distribution cannot plausibly inoculate people as quickly as a deliberately released virus will spread: the omicron variant spread from a single point of origin to infect 26% of Americans on the other side of the world within 100 days of detection.[6]

If many pandemic-capable viruses become known – even if each has only a moderate chance of causing a pandemic – a terrorist could assemble and release them all, potentially unleashing more and faster-spreading pandemics at the same time than would naturally occur in a century.

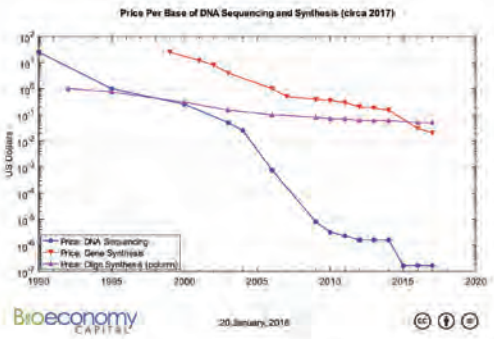


Successful pandemic virus identification will immediately cause widespread proliferation

Acquiring a pandemic-class agent requires 1) knowing of one or more viruses likely to cause a new pandemic, and 2) obtaining an infectious sample. Twenty years ago, the only way to obtain physical virus samples was from clinical specimens or laboratory stocks. **Today, thousands of individuals can assemble many types of viruses from commercially available synthetic DNA and virus assembly instructions**, often called "reverse genetics" protocols.

[5] Sewell, "Laboratory-Associated Infections and Biosafety"; Merler et al., "Containing the Accidental Laboratory Escape of Potential Pandemic Influenza Viruses"; Klotz and Sylvester, "The Consequences of a Lab Escape of a Potential Pandemic Pathogen"; Lipsitch and Inglesby, "Moratorium on Research Intended to Create Novel Potential Pandemic Pathogens"; Gryphon Scientific, "Risk and Benefit Analysis of Gain of Function Research"; Manheim and Lewis, "High-Risk Human-Caused Pathogen Exposure Events from 1975-2016"; Bloom et al., "Investigate the Origins of COVID-19."
[6] Clarke et al., "Seroprevalence of Infection-Induced SARS-CoV-2 Antibodies - United States, September 2021-February 2022."

Ingredient 1: Inexpensive Synthetic DNA



In 2002, poliovirus was successfully assembled from chemically synthesized DNA.[7] Since then, the cost of synthetic genes has fallen by a factor of a thousand.

The members of the International Gene Synthesis Consortium, an industry group, have taken the lead in voluntarily screening customer orders for dangerous agents at their own expense, going well beyond the weak regulatory requirements imposed by the Department of Health and Human Services.[8] However, members comprise only an estimated 80% of the market and the membership list is publicly available.[9]

Despite the best efforts of the International Gene Synthesis Consortium, it is currently easy to obtain unscreened synthetic DNA.

Ingredient 2: Virus assembly protocols that can be performed by individuals with little tacit knowledge

Meanwhile, **virus assembly instructions have been developed for nearly all families of viruses to facilitate research on treatments**[10]. For well-studied viral subfamilies, these step-by-step protocols are so detailed that they can be successfully performed by non-specialists with basic molecular or cellular biology skills, as long as their skillset includes mammalian cell culture: the protocols are designed to remove any need for "tacit knowledge". A recently published protocol to engineer coronaviruses such as SARS-1 and SARS-2 explicitly stated that it aimed to "enable researchers from different research backgrounds to master the use of the reverse genetic system".[11]

Ingredient 3: Many individuals with the technical skills and resources needed to succeed at virus assembly

A large number of scientists, engineers, and lab technicians have the skills required to obtain many types of infectious viruses from publicly available genome sequences. Most presumably have access to laboratory facilities due to their professional work, but even if not, **a laboratory stocked with the relevant used equipment is affordable on an upper-middle-class salary in most developed nations.**

[7] Cello, Paul, and Wimmer, "Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template."
[8] Diggans and Leproust, "Next Steps for Access to Safe, Secure DNA Synthesis"; International Gene Synthesis Consortium, "Harmonized Screening Protocol V2."
[9] "International Gene Synthesis Consortium"; Johns Hopkins Center for Health Security, "Gene Synthesis Companies."
[10] Maroun et al., "Designing and Building Oncolytic Viruses."
[11] Xie et al., "Engineering SARS-CoV-2 Using a Reverse Genetic System."

How many people can build viruses? In the U.S. alone, twenty-five new individuals receive their doctorate in the life sciences or bioengineering each day[12]; in the last 30 years, over two million people have received an equivalent degree worldwide per OECD records[13]. Even assuming that only one in ten received any training in mammalian cell culture – which is especially common among biomedical researchers – and that just one in twenty of the remainder are skilled and well-practiced enough to successfully follow a virus assembly protocol, over 10,000 people with doctorates can generate many known viruses from families for which a relevant assembly protocol is available. The number of research technicians and students may be comparable.

Another way to estimate the number is to consider how many PhDs are granted in virology, as the vast majority of such individuals must be presumed capable of following a reverse genetics protocol. The U.S. grants 125 doctoral degrees in virology each year, accounting for one-third of the total worldwide. At least four times as many individuals with degrees in related fields – such as my own PhD in biochemistry – possess similar skills. If we assume a 20-year active career, **approximately 30,000 individuals are capable of assembling any influenza virus for which a genome sequence is publicly available**.

These skills are vital to the bioeconomy, which in turn is becoming essential to human health, industrial production, environmental protection, and the continued development of a flourishing and sustainable society. The number of individuals capable of single-handedly assembling viruses from synthetic DNA will continue to grow.

Ingredient 4 (missing): Credible knowledge of novel viruses that could likely cause a new pandemic

Readily available synthetic DNA, step-by-step virus assembly protocols, and thousands of technically skilled individuals add up to many individuals who can assemble viruses, but that doesn't mean we know of any likely to cause a new pandemic. Pandemic proliferation is a nascent threat that will not be realized until someone credibly identifies a novel virus that would likely spread on its own and shares the complete genome sequence.

Consider an analogy: **we live in the biological version of a world in which weapons-grade plutonium can be mail-ordered and thousands of engineers have the skills to assemble a nuclear device, but no one knows exactly which design would work**. In that alternate reality, it's hard to imagine anyone openly seeking to identify such designs and share them online. But if cities were randomly destroyed by naturally occurring nuclear explosions, no one had ever died from a deliberate detonation, and scientists suspected that understanding how it happens might help prevent some of those natural casualties, perhaps they would. Key questions include whether doing so would be worth the cost, and how it can be done.

[12] National Center for Science and Engineering Statistics, "Doctorate Recipients from U.S. Universities, 2019."
[13] OECD, "OECD: Graduates by Field."

How can we learn whether a virus would likely cause a pandemic if exposed to humans?

Malevolent actors interested in pandemic-capable viruses as a "poor man's nuke" will not bother trying to assemble one unless they are at least moderately confident that it would cause a pandemic. An estimated 10,000 to 320,000 mammalian viruses are thought capable of infecting humans.[14] But infection alone isn't enough: **only a tiny fraction of human-infecting animal viruses – certainly no more than a few thousand – might be transmissible enough to cause a pandemic**. By default, we can safely assume that most viruses are not pandemic-capable, even if they have been flagged by computational tools or mutated by researchers in the lab to increase transmissibility. For now, the only way to substantially increase our confidence that a given virus would cause a new pandemic is to perform a certain set of laboratory experiments.

Here is the logic: viruses currently circulating in humans are very good at infecting us and making our bodies churn out more viruses. But because most of us have already been infected and acquired some immunity, they mostly spread to children who have not been exposed or to people with weak immune systems. Pandemics happen when a new virus that can be readily transmitted jumps from animals to people: no one has much immunity, so each person infects more than one other on average, causing it to spread like wildfire. Once most people have encountered the new virus and developed resistance, it behaves like its human-adapted relatives: the average infected person passes on the virus exactly once.

This means that **any virus that is immunologically new to human populations and can efficiently infect our cells, replicate in our cells, and/or be transmitted between animals chosen for their similarity to humans nearly as well as a long-circulating human virus from the same family is reasonably likely to cause a pandemic**. Even if it is subpar at one or two of these, it only needs to be good enough to keep going long enough for a variant to arise with a mutation that makes it better – just as the original SARS-CoV-2 was outcompeted by subsequent more infectious and/or immune-evasive variants. In a deliberate large-scale release, such evolution would become more likely.

Scientists attempting to identify pandemic-capable respiratory viruses typically perform experiments measuring their ability to infect and replicate in primary human airway epithelial cells and to be transmitted between human-relevant animal models, such as engineered mice, ferrets, or primates. With NIH and USAID support, scientists from EcoHealth Alliance and the Wuhan Institute of Virology with performed these types of experiments on bat coronaviruses newly collected by virus hunters to learn whether they might cause new pandemics, and also on synthetic viruses created by mixing and matching those that seemed risky in order to learn whether such recombination might produce a pandemic-capable virus.[15] Researchers working to enhance the transmissibility of especially lethal animal viruses, like the bird flu strains engineered to be transmitted more efficiently between ferrets with NIH funding, also conduct these experiments to see whether mutated versions of these viruses may have acquired the ability to cause a pandemic.[16] **These pandemic virus identification experiments are the virological equivalent of nuclear testing**. As yet, none of them have succeeded in producing any credible threats.

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[14] Carlson et al., "Global Estimates of Mammalian Viral Diversity Accounting for Host Sharing"; Anthony et al., "A Strategy to Estimate Unknown Viral Diversity in Mammals."
[15] Hu et al., "Discovery of a Rich Gene Pool of Bat SARS-Related Coronaviruses Provides New Insights into the Origin of SARS Coronavirus."
[16] Herfst et al., "Airborne Transmission of Influenza A/H5N1 Virus between Ferrets"; Imai et al., "Experimental Adaptation of an Influenza H5 HA Confers Respiratory Droplet Transmission to a Reassortant H5 HA/H1N1 Virus in Ferrets."

Key experiments that can increase our confidence that a novel animal virus is pandemic-capable
1. Quantify the growth of the novel virus in relevant human primary cells (e.g. respiratory epithelial) 2. Quantify transmissibility in a human-relevant animal model (e.g. transgenic mice or ferrets)

Will pandemic virus identification save or cost lives?

Natural pandemics killed over a million people in 1889-91, 1918-20, 1957-59, 1968-70, and 2019-21. Might the alleged benefits of pandemic virus identification outweigh the expected harms of accident risks? What of the risks of deliberate misuse?

The issue of whether we should identify pandemic-capable viruses is entirely distinct from the controversy over the origin of SARS-CoV-2. Groups promoting and performing pandemic identification virus identification research – such as EcoHealth Alliance, the Wuhan Institute of Virology, and the Global Virome Project – believe that identifying a risky virus before the first cases appear will help prevent spillover by limiting human-animal contact and blocking transmission, and hope that it might also accelerate vaccine development. But many of the most vocal proponents of a natural origin for SARS-CoV-2 have vociferously argued that it will do neither.[17] These scientists fully support the other aspects of the One Health program for spillover prevention that are backed by USAID, but they view pandemic virus identification as a wasteful diversion of resources that would be better spent monitoring high-risk populations at the animal-human interface and helping those communities contain outbreaks.

To understand whether pandemic virus identification is worth the risk, we need low and high estimates of the benefits from early therapeutic development and improved spillover prevention, the likelihood of accidents that lead to outbreaks and then pandemics, and the probability of deliberate pandemics caused by terrorists who release one or many candidate viruses across multiple sites.

Pandemic virus identification will not significantly accelerate vaccine development. Moderna's SARS-CoV-2 vaccine was designed in less than two days. With suitable manufacturing facilities, we can produce enough doses in a week to run combined Phase I and II trials using ring vaccination to simultaneously maximize the chance of containment and learn whether our vaccine is effective. Early identification will not accelerate this timeline because we cannot run Phase II challenge trials of candidate vaccines in advance: doing so would require us to deliberately infect people with presumed pandemic-capable viruses of unknown lethality that have never infected a human and may never do so. Moreover, there are so many viruses in nature that the odds are strongly against identifying the one that will actually cause the next natural pandemic. With such a low expected rate of return, we are extremely unlikely to fund the development of such vaccines in the first place.[18] Broad-spectrum vaccines are different: they could be stockpiled in advance, but do not require us to know anything about which viruses could cause pandemics – after all, the entire point is to develop vaccines that work against an entire family of viruses – so their development will not benefit from pandemic virus identification.

[17] Holmes, Rambaut, and Andersen, "Pandemics: Spend on Surveillance, Not Prediction"; Wille, Geoghegan, and Holmes, "How Accurately Can We Assess Zoonotic Risk?".
[18] Holmes, Rambaut, and Andersen, "Pandemics: Spend on Surveillance, Not Prediction"; Wille, Geoghegan, and Holmes, "How Accurately Can We Assess Zoonotic Risk?"

Pandemic virus identification may help prevent spillover – *if* the virus would have spilled over. Since there are many more pandemic-capable viruses in animals than there are severe pandemics in a century – likely at least 100-fold more – even perfect prevention of a successfully identified virus would on average prevent only 1/100 of a pandemic. Still, for a virus equivalent to SARS-CoV-2, that would save an expected 10,000 American lives. There is a reason that many scientists think it worth trying.

The risk of an accidental pandemic may or may not outweigh the benefits of identification. Lab accidents happen routinely, but estimates of the frequency and the likelihood that they would prove infectious enough to seed a pandemic (1-30%) vary enough that the expected number of lives to accidental pandemics could be lower or higher than the lives saved through spillover prevention.[19] Perhaps surprisingly, learning the true origin of SARS-CoV-2 would barely budge these numbers.

Deliberate pandemics are expected to kill many more people than identification could save. While the possibility of deliberate pandemics has seldom been raised, simple risk calculations are straightforward.[20] Given the known existence of capable mass-murderers such as Seiichi Endo of the apocalyptic cult Aum Shinrikyo, the Aurora shooter, and the Unabomber, all of whom had or plausibly could have obtained the skills to assemble and release a virus if they lived today[21], it would be surprising if the chance that any given identified virus will be released by a terrorist was less than 1% per year… meaning each identified virus would be released within a century. Because a virus deliberately introduced at multiple sites would spread more rapidly than if the same virus were to cause a natural pandemic, successfully identifying a new equivalent of SARS-CoV-2 and sharing its genome with the world is expected to cost well over a million American lives.

Even if identifying a pandemic-capable virus in nature allowed us to perfectly prevent that virus from causing a natural pandemic, and we could do so with zero risk of accidents, we should expect terrorists to use the same virus to kill a hundred times as many Americans as would be saved.

Will malicious actors identify viruses capable of causing new pandemics if we do not?

Judging by the history of nuclear weapons, many will argue that malevolent actors will eventually identify pandemics on their own, so it's better if the good guys do it first. That may have been true of the atom bomb, but the strategic calculus for pandemics could not be more different. **Malicious actors are exceedingly unlikely to identify pandemic-capable viruses if health agencies decline to do so.**

First, it's true that rogue nations and extremist groups could gain tremendous coercive power by possessing viruses understood to be capable of causing new pandemics. These could serve as "dead-hand" switches for autocratic regimes or as convenient sources of leverage for extremist groups making demands. But neither type of actor wants to actually release such an agent, let alone give access to ideological zealots who would use it to kill them.

[19] Klotz and Sylvester, "The Consequences of a Lab Escape of a Potential Pandemic Pathogen"; Gryphon Scientific, "Risk and Benefit Analysis of Gain of Function Research"; Dobson et al., "Ecology and Economics for Pandemic Prevention."
[20] Inglesby and Relman, "How Likely Is It That Biological Agents Will Be Used Deliberately to Cause Widespread Harm? Policymakers and Scientists Need to Take Seriously the Possibility That Potential Pandemic Pathogens Will Be Misused"; Katz et al., "Mapping Stakeholders and Policies in Response to Deliberate Biological Events"; "A Spreading Plague: Lessons and Recommendations for Responding to a Deliberate Biological Event"; Sandberg and Nelson, "Who Should We Fear More: Biohackers, Disgruntled Postdocs, or Bad Governments? A Simple Risk Chain Model of Biorisk."
[21] Levy and Smithson, "Ataxia: The Chemical and Biological Terrorism Threat and the US Response"; Wikipedia contributors, "James Holmes (mass murderer)"; Kaczynski, "The Unabomber Manifesto: Industrial Society and Its Future."

Second, whereas nuclear tests yield unmistakable seismological signatures of success or failure, experimental data indicating pandemic capability is easily fabricated. Even if a rogue state or extremist group were to threaten to release a pandemic, there is no reason to believe they have a capable virus, nor to accept the validity of any alleged experimental results they might provide. Threats from rogue actors will only be taken seriously if more trustworthy actors have independently identified the virus as likely to cause a pandemic. **Since credible pandemic virus identification would give access to every ideological terrorist intent on mass murder, it is against the strategic interests of every non-suicidal malicious actor** – but if well-meaning scientists unaware or dismissive of security identify it first, many non-state actors will presumably make threats with their newly credible nuclear-equivalent capabilities.

Third, pandemic virus identification experiments are far more difficult to perform than virus assembly. While the modern equivalent of a trained mass murderer such as Seiichi Endo could unquestionably assemble many viruses, they lack the technical capability to perform basic science research at the scale needed to find the pandemic needle in the animal virus haystack. Despite recent technological advances that have made success more likely, professional scientists funded by NIAID and USAID's PREDICT program have already spent hundreds of millions of taxpayer dollars searching for pandemic viruses without finding any truly credible threats. Therefore, **while bioterrorist zealots could assemble pandemic-capable viruses that health researchers helpfully identify for them, they could not find such viruses on their own.**

Most importantly, the security agencies of established nations can be persuaded that pandemic virus identification is not in their strategic interest. Pandemic-class agents kill indiscriminately and cannot currently be engineered to spare one's own population. Large nations that attempt to vaccinate their own populations in advance would likely be discovered by foreign intelligence agencies, and even if population-specific targeting became possible, its use by a nation-state would be so obvious as to invite mass retaliation. Therefore, **pandemic-capable viruses offer little if any strategic utility to powerful nation-states; indeed, quite the opposite.** The United States, China, and even Russia have a shared interest in joining forces to prevent rogue actors and terrorist zealots from gaining access to pandemic-capable viruses.

If the United States, which historically has been the largest backer of pandemic virus identification, halts such research and explains why, even our strategic rivals are likely to follow suit.

Regulatory reforms that could prevent proliferation

COVID-19 demonstrated that we remain profoundly vulnerable to pandemic viruses spreading outwards from a single point of introduction. There is no question that would fail to contain multiple pandemic agents simultaneously released in travel hubs. But multiple new technologies need not remain helpless.

Recommendation I – announce findings, redirect funds away from pandemic virus ID, and fix oversight

Our best current defense against pandemic weapons of mass destruction is to keep them from being developed in the first place.

- First, **Congress can publicly assess the threat and release a clear finding.** A Congressional finding that experiments capable of increasing public confidence that a particular virus could cause a pandemic threatens national and international security would prove instructive for security and health agencies as well as the State Department.

- Second, **Congress can stop funding pandemic virus identification experiments.** Existing programs, whether focused on naturally collected viruses or those generated by so-called "gain-of-function" research aiming to increase transmissibility, are primarily funded by governments, including our own. I deeply respect the researchers working on these programs, who have dedicated their lives to preventing natural pandemics. I do not expect them to have carefully considered the possibility of misuse.[22] Scientists are not incentivized to become security experts; even those with an interest are unlikely to be aware of many details relevant to proliferation, such as falling gene synthesis costs, the current efficacy of DNA synthesis screening, the obviating of tacit knowledge requirements by modern virus assembly protocols, the history of nuclear weaponry, and strategic game theory.

  USAID has already pledged to cease funding transmissibility enhancement ("gain-of-function") experiments, and may not have become aware of the security implications of identifying natural pandemic-capable viruses until late 2021. Even so, they remain the largest funder of such efforts, including a new program, "DEEP VZN", that is the direct successor of the PREDICT program that funded the pandemic virus identification research in Wuhan. Because these are offshoots of larger One Health programs focused on useful virus discovery and monitoring work at the animal-human interface,[23] there would be no need to revoke any funding or break contracts: the DEEP VZN program could simply direct funds towards early warning systems rather than laboratory pandemic virus characterization. They should also refrain from publicly sharing the entire genome sequences of newly discovered viruses, as much of the genome is not relevant to vaccine or antiviral therapeutic development. USAID's "STOP SPILLOVER" program need only announce that it will no longer maintain a list of viruses rank-ordered by perceived threat level[24] due to national security and proliferation risks. Behavioral studies and public health interventions, which are important for reducing the spillover of animal pathogens into human populations and containing them when possible, can and should continue.[25] Pandemic virus identification experiments represent considerably less than 1% of all virology; banning them would be much less of an imposition on the field than are the security measures governing nuclear physics.

  NIH has a long history of funding projects aiming to enhance the transmissibility of viruses, including but not limited to lethal pathogens such as H5N1 and MERS. Many of these projects, which have been compiled and summarized by reporters from the *Washington Post*, were not covered by the moratorium on "gain-of-function" research due to disputes over what exactly is meant by that term.[26] **Congress can resolve the confusion over definitions by blocking federal funding of pandemic virus identification experiments, defined as those that could substantially increase our confidence that a virus would cause a pandemic if repeatedly introduced.**

[22] "Opportunities Exist for the National Institutes of Health To Strengthen Controls in Place To Permit and Monitor Access to Its Sensitive Data."
[23] "WSU to Lead USAID's Global Sampling Project for Discovery of Emerging Viral Zoonoses - Global Biodefense"; "STOP Spillover."
[24] "SpillOver — Global Virome Project"; Grange et al., "Ranking the Risk of Animal-to-Human Spillover for Newly Discovered Viruses"; "STOP Spillover."
[25] Saylors et al., "Socializing One Health: An Innovative Strategy to Investigate Social and Behavioral Risks of Emerging Viral Threats.".
[26] Willman and Muller, "A Science in the Shadows."

Experiments that may increase our confidence that a virus is pandemic-capable
1. The comparative growth of an animal virus or chimera in relevant human primary cells 2. The comparative transmission of an animal virus in a human-relevant animal model 3. The capacity of an engineered human virus* to evade innate immunity 4. The capacity of an engineered human virus* to evade pre-existing humoral immunity 5. The capacity of an engineered human virus* to evade pre-existing cellular immunity
* This category would not include viral mutants and variants thought to already be present in nature.

- **Third, Congress can require external security oversight of life sciences research.** At present, health agencies and funding recipients are instructed to regulate themselves with respect to security issues:

  > "The Department of Health and Human Services (HHS) Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens is intended to guide HHS funding decisions…"
  >
  > "Funders of life sciences research and the institutions and scientists who receive those funds have a shared responsibility for oversight of DURC (dual use research of concern) and for promoting the responsible conduct and communication of such research."

  No funding agency or recipient can be expected to oversee itself; that is the definition of a conflict of interest. The National Science Advisory Board on Biosecurity (NSABB) consists of ostensibly independent researchers, but most are primarily funded by NIH, they are appointed by HHS, and they can be dismissed at any time. Indeed, 11 of the inaugural members who had participated in discussions over research enhancing the transmissibility of highly lethal H5N1 influenza were summarily dismissed in 2014 amidst a controversy over laboratory safety mishaps involving smallpox, influenza, and anthrax. During the vote on whether to discontinue the moratorium on "gain-of-function" research involving potential pandemic pathogens, the directors of the NIH and the NIAID, whose opinions on the matter are well-known, were allegedly present in the room.

  Congress can establish a panel of experts from security agencies to provide oversight for life sciences research funded by the U.S. government, including reviewing and approving all requests for proposals before they are released. Members should be required to recuse themselves from oversight of proposals from their own agencies.

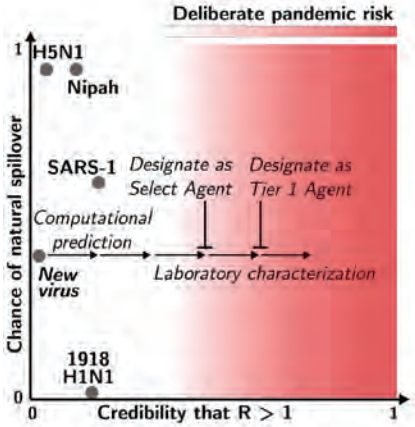
Recommendation II – Amend the 2002 Bioterrorism Response Act to update the Select Agent program

The Federal Select Agent & Toxin Program (FSAP) is unique in regulating all research in the United States, not just federally-funded entities, and additionally impacts the export control list. However, it is updated slowly, doesn't include most viruses that might be pandemic-capable, and the Act was last amended before we developed techniques such as virus chimerism, directed evolution, ancestral protein reconstruction, and machine learning approaches, all of which can plausibly generate new threats from existing ones.

Congress can update the Federal Select Agent & Toxin Program to:

1. Automatically list a virus as soon as a single result from a pandemic virus identification experiment suggests that it may be pandemic-capable[27]
2. Regulate any DNA construct that was generated from pieces of regulated Select Agents
3. Give the program the power to immediately lift all restrictions on any Select Agent confirmed to be actively spreading in order to accelerate research on countermeasures

In addition to reducing accident risks and requiring background checks of anyone working with viruses that might cause new pandemics – clearly the most hazardous of biological agents – these rules would disincentivize researchers from performing experiments to determine whether a virus they study is pandemic-capable, as doing so would automatically render it a Select Agent and increase their laboratory's cost of research several fold. As a gesture of good faith to the scientific community, FSAP might also be instructed to consider removing many listed agents that are incapable of autonomous spread and pose comparatively little risk of deliberate misuse.



Assessing pandemic risks. Pandemics may result from natural spillover, laboratory accidents, or deliberate misuse of viruses identified as credibly pandemic-capable (basic reproductive number R>1: the typical infected person infects more than one other person). Designating viruses as Select Agents upon obtaining the first experimental evidence indicative of pandemic potential could preserve the hypothesized benefits of virus discovery for "universal" virus family vaccine and broad-spectrum antiviral development while reducing accident risks and deterring characterization experiments that would otherwise result in the proliferation of viruses with presumed nuclear-equivalent lethality.

[27] **Defining pandemic-capable:** Any virus that normally circulates in a population (R~1) will cause a pandemic when introduced into a more susceptible population that lacks pre-existing immunity (R>1). This is why pandemics typically arise from viruses that spill over from other species, which spread rapidly before becoming endemic. Therefore, a virus is a credible pandemic threat if it:

- Can grow in relevant human tissues or be transmitted between relevant animal models nearly as well an endemic human virus of the same family
- Is poorly recognized by the immune systems of most humans relative to endemic human viruses of the same family

Recommendation III – leverage shared strategic interests to prevent pandemic proliferation globally

The nature of many emerging technologies places the U.S. and China at loggerheads, but our strategic interests are nearly perfectly aligned when it comes to the proliferation of access to pandemic viruses: both nations have little to gain and much to lose. This is an opportunity for the United States to gain leverage by offering information exchange and inviting co-leadership in global health security, and may help build diplomatic channels to address more challenging issues around other key technologies.

One way to marshal global action against pandemic virus prediction would utilize the Biological Weapons Convention (BWC), which prohibits the "development, stockpiling, acquisition, retention and production of biological agents" while "permitting the fullest possible exchange of equipment, materials, and information for peaceful purposes." Today, it's impossible to identify a credible pandemic-capable virus from most families, including corona-, influenza- and paramyxoviruses, without immediately giving thousands of individuals the ability to assemble infectious samples. There is a strong argument that Article III compels BWC signatories to block pandemic virus identification despite the good-intentions provisions of Article X.[28] However, the BWC is normatively important, but too general and without enforcement provisions to address pandemic proliferation. Instead, **Congress can instruct the State Department to begin negotiating a Pandemic Test-Ban Treaty that would narrowly forbid pandemic virus identification experiments, defined as those that could substantially increase our confidence that an animal virus or an engineered virus could cause a new pandemic.**

Recommendation IV – require DNA synthesis screening matching or exceeding the industry standard

Most researchers who can follow a virus assembly protocol cannot synthesize and assemble the requisite DNA or RNA, so the fact that we can order synthetic viral DNA and have it come in the mail substantially increases the number of actors capable of assembling a pandemic weapon. California's legislature passed a bill that would require all providers of synthetic DNA and manufacturers of synthesis machines to screen orders at least as well as the International Gene Synthesis Consortium, but it was vetoed on the grounds that federal legislation is needed to avoid a regulatory patchwork.[29]

A federal law requiring DNA synthesis screening would pressure domestic and international providers to screen, nudging firms to engage with the NTI/WEF stakeholder discussions on a cooperative framework and the SecureDNA project to implement new advances, including in "desktop" synthesizers, that will allow automated screening for the latest threats without having to disclose customer orders or jeopardize trade secrets.[30] Similar regulations could be encouraged internationally using the BWC or other diplomatic means.

[28] Butler, "Bioweapons Treaty in Disarray as US Blocks Plans for Verification."
[29] "California Legislature - AB-70 Gene Synthesis Providers."
[30] "Biosecurity Innovation and Risk Reduction: A Global Framework for Accessible, Safe and Secure DNA Synthesis"; The SecureDNA team, "Secure DNA Project - DNA Synthesis Screening."

Recommendation V – implement catastrophe liability and require insurance coverage

Despite the recently demonstrated catastrophic potential of pandemics and the controversy over accident risks, there have been surprisingly few professional risk assessments. Indeed, the United States imposed a moratorium on poorly-defined "gain-of-function" research, removed it, and replaced it with an equally poorly defined and readily evaded framework governing research on "enhanced potential pandemic pathogens" without any publication of a quantitative risk model.

Pandemic virus identification may offer great rewards – preventing 10,000 American deaths would be a major accomplishment – but there is currently no way to assess the negative externalities, let alone any market-based way to incorporate those costs into the decision-making process. With the narrow exception of nuclear policy, other actions with potentially catastrophic consequences are similarly unregulated.

Congress can encourage market-based professional risk assessment of potential catastrophes by:

1. **Passing legislation to ensure that institutions causally linked to catastrophic outcomes involving the death or disablement of a million or more Americans can be held legally liable, even for providing information permitting deliberate misuse by others, and**
2. **Requiring that general liability insurance cover such claims, up to a very large market cap chosen to prevent insurers and re-insurers from going bankrupt while providing a very strong incentive to evaluate potential sources of catastrophic risks**

The Covid-19 pandemic is the only historical event to have caused a million American deaths. World War I, World War II, HIV, the 1918 influenza pandemic, and even the Civil War did not claim so many.[51] This inherently limits the number of actions that would require review. Not only is it entirely reasonable to hold responsible anyone whose actions are subsequently shown – through the due process of law – to be instrumental in the genesis of such catastrophes, but requiring general liability insurance to cover that liability would effectively require professional risk analysts to evaluate the likelihood and set rates accordingly. That is, such a law would induce the market to impose costs upon institutions proportional to the available information concerning the likelihood that their current and planned actions will, at even very low probabilities, lead to extreme catastrophe. Requiring any findings above a minimal expected consequence (for example, probability x magnitude > 10,000 American deaths) to be made public following review and potential classification by a panel of security experts would add to society's understanding of which actions might trigger catastrophe, including by improving future assessments of the same type by insurers and prediction markets.

If a catastrophe liability and insurance act already existed, there might not be a need for Congress to determine whether to ban pandemic virus identification experiments: simply informing the insurance companies covering the institutions involved would trigger professional assessment and a corresponding increase in premiums, forcing the involved institutions to internally evaluate whether to continue with the risky behavior. If deemed worth the cost, then such actions would proceed. If not, the problem would solve itself. Either way, the negative externalities of actions with sufficiently low-probability and high-consequence outcomes would be incorporated into decision-making.

[51] Roos, "The Deadliest Events in US History."

Congressional regulation can buy time sufficient to render the U.S. able to withstand pandemics

There is a saying in cybersecurity: any system vulnerable to accidents is helpless against deliberate attack. Covid-19 may have arisen from nature or the accidental infection of a virus hunter or laboratory researcher, but it was unquestionably an accident. It follows that we are currently helpless against deliberate attacks involving pandemic-class agents. **Our current vulnerability to pandemics underscores the vital importance of ensuring that blueprints do not become publicly available.**

But if our vulnerability is extreme enough to make the situation seem hopeless, it might impede productive efforts to take action: there is a natural human inclination to freeze when confronted with a seemingly intractable problem. The deliberate release of pandemic viruses may well threaten the United States in ways that cannot be solved by medical countermeasures due to logistical constraints, no matter how quickly developed. This does not mean pandemics represent an insoluble problem. **We can immunize our country against pandemic events within a decade without any advances in biomedicine.**[32]

New algorithms are making it possible to automatically screen all DNA synthesis without revealing which sequences are considered hazardous, reducing unauthorized access to pandemic-class threats by a hundredfold and nudging scientists away from publicly disclosing them in the first place.[33] Advances in DNA sequencing will provide the United States with reliable early warning of all exponentially spreading biological threats, even those such as HIV that would not immediately show up in the clinic, for under a billion dollars a year.[34] Early warning will allow us to provide essential workers with protective equipment sufficient to enable them to do their jobs without risking their lives – an N95 mask is unlikely to suffice for a SARS-CoV-2-equivalent virus with 30% lethality, let alone something as contagious as measles, but slightly improved powered air-purifying respirators would – and enabling us to contain and eliminate threats even if groups of pandemic viruses are released in multiple airports. Personalized risk assessments provided by improved versions of contact tracing apps could tell people what fraction of their first-, second-, third-, fourth, and fifth-degree contacts have been infected, allowing only those at-risk to take precautions and dramatically reducing the cost of extinguishing small outbreaks brought in from outside our borders.[35] Perhaps most importantly, we're learning that certain wavelengths of light can kill viruses and pathogenic bacteria without even penetrating our skin or eyes; if we determine that they are as safe as preliminary studies indicate and install them in fixtures throughout the country, we could plausibly suppress a future Covid-19 or possibly even a measles-class pandemic without anyone having to wear a mask or change their routine at all… and virtually eliminate the common cold and flu while we're at it.[36]

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[32] Esvelt, "Delay, Detect, Defend: Preparing for a future in which tens of thousands can unleash new pandemics." Unpublished; draft available upon request.
[33] The SecureDNA team, "Secure DNA Project - DNA Synthesis Screening"; Gretton D, Wang B, Foner L, DeBenedictis EA, Liu AB, Chory E, Cui H, Li X, Dong J, Fabrega A, Dennison C, Don O, Tong Y, Uberoy K, Rivest R, Gao M, Yu Y, Baum C, Damgard I, Yao AC, Esvelt KM, "Random Adversarial Threshold Search Enables Specific, Secure, and Automated DNA Synthesis Screening"; The SecureDNA cryptography team, "Hiding Dangerous DNA in Plain Sight."
[34] The Nucleic Acid Observatory Consortium, "A Global Nucleic Acid Observatory for Biodefense and Planetary Health."
[35] Loh, "NOVID - a New Approach to Pandemics"; Loh, "Flipping the Perspective in Contact Tracing."
[36] Blatchley et al., "Far UV-C Radiation: An Emerging Tool for Pandemic Control."

Developing and implementing these defenses will take time. Without action by Congress, the first highly credible pandemic viruses might be publicly identified and their complete genomes irreversibly shared by well-meaning scientists funded by USAID, NIH, or other agencies as soon as this coming year. We need to keep the risk window closed for as long as possible. Disagreements over public health policy, accidents, and the origin of SARS-CoV-2 appear trivial next to this emerging technological threat to national security.

For 75 years, the United States has successfully kept nuclear capabilities out of the hands of terrorists. Today, we're on the verge of irreversibly handing them blueprints for viruses as lethal as nuclear weapons – all in the name of public health. Let's not.

This testimony reflects the personal opinions and technical expertise of Dr. Kevin M. Esvelt.
Dr. Esvelt is currently a professor at MIT, but does not speak on behalf of the Institute on this occasion.

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July 14, 2021

The Honorable Rosa DeLauro
Chair
House Committee on Appropriations
U.S. House of Representatives
Washington, DC 20515

The Honorable Kay Granger
Ranking Member
House Committee on Appropriations
U.S. House of Representatives
Washington, DC 20515

The Honorable Tom Cole
Ranking Member
Subcommittee on Labor, Health and Human Services, Education
U.S. House of Representatives
Washington, DC 20515

Dear Chair DeLauro, Ranking Member Granger and Ranking Member Cole:

As Congress exercises its oversight authority over the response to the COVID-19 pandemic, we urge you to lead with science. Scientists, who have the appropriate subject-matter expertise, provide the best opportunity to answer key questions regarding the origin and transmission of the pandemic. Congress should follow an evidence-based process to ensure answers are grounded in sound science, are as complete as possible, and based in fact.

We caution against adopting policy changes absent scientific evidence, because doing so could further undermine the public's confidence in science. For future scientific progress, the implications of such proposals extend far beyond the immediate challenge of the pandemic.

We urge you to reject attempts to impose restrictions on federally funded research or the operations of federal science agencies based on premature conclusions about how the pandemic emerged. Such efforts could have serious, negative unintended consequences for potentially lifesaving research. They would harm the very ecosystem that developed the novel tests, vaccines, and medical countermeasures that have brought us through the pandemic. Continued support for research is essential for tackling a number of society's most vexing challenges, including combatting emerging infectious diseases and preventing the next pandemic.

On behalf of the scientists and clinicians our organizations and institutions represent, we urge you to allow the scientific process to play out as we seek to understand how the pandemic

began and how we can protect ourselves from future threats to our nation's health and economy.

Sincerely,

American Association for Anatomy
American Association of Veterinary Medical Colleges
American Dairy Science Association
American Geophysical Union (AGU)
American Institute of Biological Sciences
American Society for Biochemistry and Molecular Biology
American Society for Investigative Pathology
American Society for Microbiology
American Society for Virology
American Society of Tropical Medicine and Hygiene
Association for Professionals in Infection Control and Epidemiology
Association for Psychological Science
Association of American Cancer Institutes
Association of American Medical Colleges
Biophysical Society
Coalition for the Life Sciences
Columbia University Irving Medical Center
Endocrine Society
Entomological Society of America
FASS
HIV Medicine Association
Institute of Food Technologists
International Society on Thrombosis and Haemostasis (ISTH)
KidneyCAN
National Association for Biomedical Research
Pediatric Infectious Diseases Society
ResearchAmerica
Rochester Institute of Technology
Society of Nuclear Medicine and Molecular Imaging
Society of Toxicology
The Gerontological Society of America
The Society for Healthcare Epidemiology of America
The Society for Pediatric Radiology
Veterans for Common Sense
Wayne State University
Yerkes National Primate Research Center, Emory University

April 8, 2022

The Honorable Nancy Pelosi
Speaker
U.S. House of Representatives
Washington, DC 20515

The Honorable Kevin McCarthy
Republican Leader
U.S. House of Representatives
Washington, DC 20515

The Honorable Chuck Schumer
Majority Leader
U.S. Senate
Washington, DC 20510

The Honorable Mitch McConnell
Minority Leader
U.S. Senate
Washington, DC 20510

Dear Speaker Pelosi, Leader McCarthy, Leader Schumer and Leader McConnell:

The undersigned organizations appreciate Congress' long-standing bipartisan support for biomedical research. We also respect that oversight of research with biosafety and national security ramifications is in the best of interest of science and the nation.

As Congress negotiates far-reaching legislation focused on advancing American competitiveness through a stronger investment in federal science agencies and programs, **we urge Congress to remove legislative provisions that would restrict, pause, or alter federally funded research projects that focus on gain of function research of concern or specific pathogens.** Such an approach through legislation is overly proscriptive and interferes with the National Science Advisory Board for Biosecurity's (NSABB) evaluation of and forthcoming recommendations on enhanced potential pandemic pathogen research (EPPP) and dual use research of concern (DURC).

Established to review and advise the federal government on biosafety and biosecurity in research, including DURC and more recently, EPPP research, NSABB has the requisite scientific expertise to address these issues. Its work is essential to formulating the most effective policies for the future as we move beyond the COVID-19 pandemic and prepare for future seasonal and pandemic threats. The NSABB convened on February 28, 2022 to discuss a new, expanded charge and the path forward.

We believe it is appropriate for these polices and frameworks to be re-evaluated considering lessons learned in the current pandemic and with an eye toward both international engagement and the appropriate balance between biosecurity and the lifesaving value of this research. These efforts include the NSABB revision of definitions and consideration of pathogens classified under DURC and EPPP, and appropriately assessing research benefits with risks.

We also believe the process' inclusion of public and stakeholder input is a critical component to this evaluation because ensuring an appropriate degree of transparency in carrying out the process is important to building trust with the public and the scientific community.

Through collaboration with NSABB, Congress can strike an appropriate balance between safeguarding national security and public health through biosafety and biosecurity measures, while also recognizing the global impact of potential pandemic pathogens, the value and lifesaving potential of research in this area, and the need to study these microbes to address current and future threats. Doing otherwise could have serious, negative unintended consequences for potentially lifesaving research. They would harm the very ecosystem that developed the novel tests, vaccines, and medical countermeasures that have brought us through the pandemic.

We encourage Congress to continue exercising its oversight responsibilities and avoid legislative provisions that would pause or even halt research projects focused on specific viruses, ban specific techniques, or restrict collaboration in specific areas of the world. We thank you for your consideration of our views.

Sincerely,

American Institute of Biological Sciences
American Society for Microbiology
American Society for Virology
Association for Professionals in Infection Control and Epidemiology
Association of American Medical Colleges
Biophysical Society
Coalition for the Life Sciences
Duke Health
Duke University
Federation of American Societies for Experimental Biology
Infectious Diseases Society of America
Michigan State University
National Association for Biomedical Research
North American Vascular Biology Organization
Pediatric Infectious Diseases Society
Research!America
University of Louisville
University of Michigan
University of Washington

Science

RESEARCH ARTICLES

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The molecular epidemiology of multiple zoonotic origins of SARS-CoV-2

Jonathan E. Pekar[1,2]*, Andrew Magee[3], Edyth Parker[4], Niema Moshiri[5], Katherine Izhikevich[5,6], Jennifer L. Havens[1], Karthik Gangavarapu[5], Lorena Mariana Malpica Serrano[7], Alexander Crits-Christoph[8], Nathaniel L. Matteson[4], Mark Zeller[9], Joshua I. Levy[4], Jade C. Wang[9], Scott Hughes[9], Jungmin Lee[10], Heedo Park[10,11], Man-Seong Park[10,11], Katherine Ching Zi Yan[12], Raymond Tzer Pin Lin[12], Mohd Noor Mat Isa[13], Yusuf Muhammad Noor[13], Tetyana I. Vasylyeva[14], Robert F. Garry[15,16,17], Edward C. Holmes[18], Andrew Rambaut[19], Marc A. Suchard[3,20,21]*, Kristian G. Andersen[4,22]*, Michael Worobey[2]*, Joel O. Wertheim[14]*

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Understanding the circumstances that lead to pandemics is important for their prevention. Here, we analyze the genomic diversity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) early in the coronavirus disease 2019 (COVID-19) pandemic. We show that SARS-CoV-2 genomic diversity before February 2020 likely comprised only two distinct viral lineages, denoted A and B. Phylodynamic rooting methods, coupled with epidemic simulations, reveal that these lineages were the result of at least two separate cross-species transmission events into humans. The first zoonotic transmission likely involved lineage B viruses around 18 November 2019 (23 October–8 December), while the separate introduction of lineage A likely occurred within weeks of this event. These findings indicate that it is unlikely that SARS-CoV-2 circulated widely in humans prior to November 2019 and define the narrow window between when SARS-CoV-2 first jumped into humans and when the first cases of COVID-19 were reported. As with other coronaviruses, SARS-CoV-2 emergence likely resulted from multiple zoonotic events.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease 19 (COVID-19) pandemic that caused more than 5 million confirmed deaths in the two years following its detection at the Huanan Seafood Wholesale Market (hereafter the 'Huanan market') in December 2019 in Wuhan, China (*1–3*). As the original outbreak spread to other countries, the diversity of SARS-CoV-2 quickly increased and led to the emergence of multiple variants of concern, but the beginning of the pandemic was marked by two major lineages denoted 'A' and 'B' (*4*).

Lineage B has been the most common throughout the pandemic and includes all eleven sequenced genomes from humans directly associated with the Huanan market, including the earliest sampled genome, Wuhan/IPBCAMS-WH-01/2019, and the reference genome, Wuhan/Hu-1/2019 (hereafter 'Hu-1') (*5*), sampled on 24 and 26 December 2019, respectively. The earliest lineage A viruses, Wuhan/IME-WH01/2019 and Wuhan/WH04/2020, were sampled on 30 December 2019 and 5 January 2020, respectively (*6*). Lineage A differs from lineage B by two nucleotide substitutions, C8782T and T28144C, which are also found in related coronaviruses from *Rhinolophus* bats (*4*), the presumed host reservoir (*7*). Lineage B viruses have a 'C/T' pattern at these key sites (C8782, T28144), whereas lineage A viruses have a 'T/C' pattern (C8782T, T28144C). The earliest lineage A genomes from humans lack a direct epidemiological connection to the

Huanan market, but were sampled from individuals who lived or had recently stayed close to the market (*8*). It has been hypothesized that lineages A and B emerged separately (*9*), but 'C/C' and 'T/T' genomes intermediate to lineages A and B present a challenge to that hypothesis, as their existence suggests within-human evolution of one lineage toward the other via a transitional form.

Questions about these lineages remain: if lineage B viruses are more distantly related to sarbecoviruses from *Rhinolophus* bats, (i) why were lineage B viruses detected earlier than lineage A viruses and (ii) why did lineage B predominate early in the pandemic?

Answering these questions requires determining the ancestral haplotype, the genomic sequence characteristics of the most recent common ancestor (MRCA) at the root of the SARS-CoV-2 phylogeny. In this study, we combined genomic and epidemiological data from early in the COVID-19 pandemic with phylodynamic models and epidemic simulations. We eliminated many of the haplotypes previously suggested as the MRCA of SARS-CoV-2 and show that the pandemic most likely began with at least two separate zoonotic transmissions starting in November 2019.

Results

Erroneous assignment of haplotypes intermediate to lineages A and B

There are 787 near-full length genomes available from lineages A and B sampled by 14 February 2020 (data S1 and S2). However, there are also 20 genomes of intermediate haplotypes from this period containing either T28144C or C8782T but not both mutations: C/C or T/T, respectively.

We identified numerous instances of C/C and T/T genomes sharing rare mutations with lineage A or lineage B viruses, often sequenced in the same laboratory, indicating these intermediate genomes are likely artifacts of contamination or bioinformatics (*10*), similar to findings from our analysis of the emergence of SARS-CoV-2 in North America (*11*) (fig. S1 and supplementary text). We confirmed that a C/C genome from South Korea sharing three such mutations had low sequencing depth at position 28144 (≤10x), a T/T genome sampled in Singapore had low coverage at both 8782 and 28144 (≤10x), and three T/T genomes sampled in Wuhan had low sequencing depth and indeterminate nucleotide assignment at position 8782 (table S1). Further, the authors of eleven C/C genomes sampled in Wuhan and Sichuan confirmed that low sequencing depth at position 8782 led to the erroneous assignment of intermediate haplotypes.

C/C and T/T genomes continue to be observed throughout the pandemic as a result of convergent evolution, including T/T aboard the Diamond Princess cruise ship outbreak and subsequent COVID-19 waves in New York City and San Diego (fig. S2 to S5 and supplementary text). Instances of convergent evolution are identifiable because SARS-CoV-2 phylogenies exist in 'near-perfect' tree space where topology can be inferred with high accuracy (*12*). These findings cast doubt on the claim that transitional C/C or T/T haplotypes between lineages A and B circulated in humans, reopening the door to the hypothesis that lineages A and B represent separate zoonotic introductions.

Progenitor genome reconstruction

To better understand SARS-CoV-2 mutational patterns, we reconstructed the genome of a hypothetical progenitor of SARS-CoV-2. Using maximum likelihood ancestral state reconstruction across 15 non-recombinant regions of SARS-CoV-2 and closely related sarbecovirus genomes sampled from bats and pangolins (*13*), we inferred the genome of this recombinant common ancestor ("recCA") (figs. S6 and S7 and supplementary text). The recCA differed from Hu-1 by just 381 substitutions, including C8782T and T28144C. It is more informative than an outgroup sarbecovirus because it accounts for the closest relative across all recombinant segments (figs. S8 to S14 and supplementary text) (*14*), and, as an internal node on the phylogeny, is more genetically similar to SARS-CoV-2 than any extant sarbecovirus.

Reversions across the early pandemic phylogeny

The ubiquity of SARS-CoV-2 reversions (*i.e.*, mutations from Hu-1 toward the recCA) indicates that genetic similarity to related viruses is a poor proxy for the ancestral haplotype. We observe 23 unique reversions and 631 unique substitutions (excluding reversions) across the SARS-CoV-2 phylogeny from the COVID-19 pandemic up to 14 February 2020 (Fig. 1). Substitutions were overrepresented at the 381 sites separating the recCA from Hu-1 (23/381 = 6.04%), compared with substitutions at all other sites (631/29,134 = 2.17%).

Most reversions were C-to-T mutations (19/23 = 82.6%), matching the mutational bias of SARS-CoV-2 (*15–17*). Genomes with C-to-T reversions can be found within lineage A, including C18060T (lineage A.1; *e.g.*, WA1) and C29095T (*e.g.*, 20SF012), as well as C24023T, C25000T, C4276T, and C22747T in mid-late January and February 2020. Hence, triple revertant genomes, like WA1 and 20SF012, are neither unique nor rare. We also identified a lineage A genome (Malaysia/MKAK-CL-2020-6430/2020), sampled on 4 February 2020 from a Malaysian citizen traveling from Wuhan whose only four mutations from Hu-1 are all reversions (lineage A.1+T6025C) (Fig. 1). Therefore, no highly revertant haplotype can automatically be assumed to represent the MRCA of SARS-CoV-2, especially when these reversions are most often the result of C-to-T mutations. In fact, we continue to observe these reversion patterns throughout the pandemic, including in the emergence of WHO-named variants (figs. S15 and S16).

Inferring the MRCA of SARS-CoV-2

To infer the ancestral SARS-CoV-2 haplotype, we developed a non-reversible, random-effects substitution process model in a Bayesian phylodynamic framework that simultaneously reconstructs the underlying coalescent processes and the sequence of the MRCA of the SARS-CoV-2 phylogeny. The random-effects substitution model captures the C-to-T transition and G-to-T transversion biases (fig. S17 and supplementary text). Using this model, referred to as the unconstrained rooting (fig. S18A), we inferred the ancestral haplotype of the 787 lineage A and B genomes sampled by 14 February 2020.

Our unconstrained rooting strongly favors a lineage B or C/C ancestral haplotype and shows that a lineage A ancestral haplotype is inconsistent with the molecular clock [Bayes factor (BF) = 48.1] (Table 1). Lineage B exhibits more divergence from the root of the tree than would be expected if lineage A were the ancestral virus in humans (figs. S19 and S20). The T/T ancestral haplotype was also disfavored (BF>10), likely because of the C-to-T transition bias (fig. S17). We acknowledge that the timing of the earliest sampled lineage B genomes associated with the Huanan market could bias rooting inference toward lineage B haplotypes; however, lineage A was still disfavored after excluding all market-associated genomes (BF=11.0).

Even though sequence similarity to closely related sarbecoviruses alone is insufficient to determine the SARS-CoV-2 ancestral haplotype, this similarity can inform phylodynamic inference. Rather than rely on outgroup rooting [fig. S18B and (*18*)], we developed a rooting method that assigns the recCA as the progenitor of the inferred SARS-CoV-2 MRCA (fig. S18C). As opposed to the unconstrained rooting, the recCA root favored a lineage A haplotype over lineage B, although support for C/C was unchanged (Table 1). Our results were insensitive to the method of breakpoint identification in the recCA (supplementary text).

The A.1 and A+C29095T proposed ancestral haplotypes were strongly rejected by all the phylodynamic analyses, even when rooting with recCA or bat sarbecovirus outgroups, which include both C18060T and C29095T (Table 1 and data S3). Hence, WA1-like and 20SF012-like haplotypes cannot plausibly represent the MRCA of SARS-CoV-2 as previously suggested (*19–21*): the similarity of these genomes to the recCA is due to C-to-T reversions. Haplotypes not reported in Table 1 were similarly rejected (data S3).

We inferred the tMRCA for SARS-CoV-2 to be 11 December 2019 (95% HPD: 25 November–12 December) using unconstrained rooting. It has been suggested that a phylogenetic root in lineage A would produce an older time of most recent common ancestor (tMRCA) than a lineage B rooting (*21*). Therefore, we developed an approach to assign a haplotype as the SARS-CoV-2 MRCA and inferred the tMRCA (*i.e.*, A, B, C/C, A.1 or A+C29095T) (fig. S18D). The tMRCA was consistent with the recCA-rooted and fixed ancestral haplotype analyses (table S2 and supplementary text).

We infer only three plausible ancestral haplotypes: lineage A, lineage B, and C/C. However, the inability to reconcile the molecular clock at the outset of the COVID-19 pandemic with a lineage A ancestor without information from related sarbecoviruses (*e.g.*, the recCA) requires us to question the assumption that both lineages A and B resulted from a single introduction.

Separate introductions of lineages A and B

We next sought to determine whether a single introduction from one of the plausible ancestral haplotypes (lineage A, lineage B, or C/C) is consistent with the SARS-CoV-2 phylogeny. We simulated SARS-CoV-2-like epidemics (*22, 23*) with a doubling time of 3.47 days [95% highest density interval (HDI) across simulations: 1.35–5.44] (*24–26*) to account for the rapid spread of SARS-CoV-2 before it was identified as the etiological agent of COVID-19 (figs. S21 and S22, tables S3 and S4, and supplementary text). We then simulated coalescent processes and viral genome evolution across these epidemics to determine how frequently we recapitulated the observed SARS-CoV-2 phylogeny.

Lineages A and B comprise 35.2% and 64.8% of the early SARS-CoV-2 genomes, and each lineage is characterized by a large polytomy (*i.e.*, many sampled lineages descending from a single node on the phylogenetic tree), with the base of lineages A and B being the two largest polytomies observed in the early pandemic (Fig. 1). Furthermore, large polytomies are characteristic of SARS-CoV-2 introductions into geographical regions at the start of the pandemic (e.g., fig. S23) (*11, 27–29*) and would similarly be expected to occur after a successful introduction of SARS-CoV-2 into humans. Congruently, the most common topology in our simulations is a large basal polytomy (with ≥100 descendent lineages), present in 47.5% of simulated epidemics (Fig. 2A).

In contrast, a topology corresponding to a single introduction of an ancestral C/C haplotype, characterized by two clades, each comprising ≥30% of the taxa, possessing a large polytomy at the base, and separated from the MRCA by one mutation (Fig. 2B), was only observed in 0.1% of our simulations. Further, a topology corresponding to a single introduction of an ancestral lineage A or lineage B haplotype, characterized by a large basal polytomy and a large clade, comprising between 30% and 70% of taxa, two mutations from the root with no intermediate genomes, was observed in only 0.5% of our simulations (Fig. 2C, see supplementary text for details).

Our epidemic simulations do not support a single introduction of SARS-CoV-2 giving rise to the observed phylogeny. We therefore quantified the relative support for two introductions resulting in the empirical topology. By synthesizing

posterior probabilities of inferred ancestral haplotypes, frequencies of topologies in epidemic simulations, and the expected relationships between these haplotypes and topologies, we infer strong support favoring separate introductions of lineages A and B (BF=61.6 and BF=60.0 using the recCA and unconstrained rooting, respectively; see Methods). This support is robust across shorter and longer doubling times, varying ascertainment rates, and minimum polytomy size (tables S4 and S5).

If lineages A and B arose from separate introductions, then the MRCA of SARS-CoV-2 was not in humans, and it is the tMRCAs of lineages A and B that are germane to the origins of SARS-CoV-2 (i.e, not the timing of their shared ancestor). Rooting with the recCA, we inferred the median tMRCA of lineage B to be 15 December (95% HPD: 5 December to 23 December) and the median tMRCA of lineage A to be 20 December (95% HPD: 5 December to 29 December) (Fig. 3A). The tMRCA of lineage B consistently predates the tMRCA of lineage A (Fig. 3B). These results are robust to using unconstrained rooting, fixing the ancestral haplotype, and excluding market-associated genomes (Fig. 3, A and B; table S2; and supplementary text).

Timing the introductions of lineages A and B

The primary case, the first human infected with a virus in an outbreak, could precede the tMRCA if basal lineages went extinct during cryptic transmission (*23*, *30*, *31*). The index case, the first identified case, is rarely also the primary case (*32*, *33*). We next used an extension of our previously published framework combining epidemic simulations and phylodynamic tMRCA inference [see Methods; (*23*, *30*, *31*)] to infer the timing of the lineage B and lineage A primary cases, accounting for both the index case symptom onset date and earliest documented COVID-19 hospitalization date.

The earliest unambiguous case of COVID-19, with symptom onset on 10 December and hospitalization on 16 December, was a seafood vendor at the Huanan market. Unfortunately no published genome is available for this case (*8*). Nonetheless, we can reasonably assume this individual had a lineage B virus (supplementary text), as an environmental sample (EPI_ISL_408512) from the stall this vendor operated was lineage B. The earliest lineage A genome (IME-WH01) is from a familial cluster where the earliest symptom onset is 15 December and earliest hospitalization is 25 December (*34*). Accounting for these dates and using the recCA rooting, we inferred the infection date of the lineage B primary case to be 18 November (95% HPD: 23 October to 8 December) and the infection date of the primary case of lineage A to be 25 November (95% HPD: 29 October to 14 December). The lineage B primary case predated that of lineage A in 64.6% of the posterior sample, by a median of 7 days (Fig. 3D and table S6).

Our lineage A and B primary case inference is robust to rooting on the recCA and fixing the plausible ancestral haplotype to lineage A, lineage B, or C/C, as well as different index case dates, accounting for only hospitalization dates, and varying growth rates and ascertainment rates (tables S7 to S10 and supplementary text). Therefore, our results indicate that lineage B was introduced into humans no earlier than late-October and likely in mid-November 2019, and the introduction of lineage A occurred within days to weeks of this event.

We then inferred the number of ascertained infections and hospitalizations arising from these separate introductions. We find that an earlier introduction of lineage B leads to a faster rise in lineage B-associated infections, dominating the simulated epidemics (Fig. 4) and recapitulating the predominance of lineage B observed in China in early 2020 (*35*). Similarly, simulated lineage B hospitalizations are more common than those from lineage A through January 2020 (fig. S24). We observe these patterns regardless of rooting strategy (unconstrained or recCA), ancestral haplotype (B, A, or C/C) (Fig. 4 and tables S11 and S12), and doubling time (figs. S25 to S28).

Minimal cryptic circulation of SARS-CoV-2

We do not see evidence for substantial cryptic circulation before December 2019 (Fig. 4), even if we assume a single introduction (fig. S29 and supplementary text). Our simulated epidemics have a median of three (95% HPD 1-18) cumulative infections at the tMRCA, with 99% of simulated epidemics resulting in at most 33 infections (table S13 and supplementary text). Further, it is unlikely there were any COVID-19 related hospitalizations before December (*36*), as the simulated epidemics show a median of zero (95% HPD: 0–2) hospitalizations by 1 December 2019. These results are in accordance with the lack of a single SARS-CoV-2-positive sample among tens of thousands of serology samples from healthy blood donors from September to December 2019 (*37*) and thousands of specimens obtained from influenza-like illness patients at Wuhan hospitals from October to December 2019 (*34*). Therefore, there was likely extremely low prevalence of SARS-CoV-2 in Wuhan before December 2019. Even when we simulated epidemics with a longer doubling time, resulting in an earlier timing of the primary cases (tables S8 and S10), there were still few infections prior to December 2019 (table S13).

Additional introductions

The extinction rate of our simulated epidemics (*i.e.*, simulations that did not produce self-sustaining transmission chains) indicate there were likely multiple failed introductions of SARS-CoV-2. Similar to our previous findings (*23*), 77.8% of simulated epidemics went extinct. These failed introductions produced a mean of 2.06 infections and 0.10

hospitalizations; hence, failed introductions could easily go unnoticed. If we treat each SARS-CoV-2 introduction, failed or successful, as a Bernoulli trial and simulate introductions until we see two successful introductions, we estimate that eight (95% HPD: 2–23) introductions led to the establishment of both lineage A and B in humans.

Limitations

Our analysis of the putative intermediate haplotypes suggests there remain lineage assignment errors between lineages A and B, particularly of genomes sampled in January and February of 2020, which could influence the precision of the phylogenetic topology and tMRCA inference. Importantly, we lack direct evidence of a virus closely related to SARS-CoV-2 in non-human mammals at the Huanan market or its supply chain. The genome sequence of a virus directly ancestral to SARS-CoV-2 would provide more precision regarding the timing of the introductions of SARS-CoV-2 into humans and the epidemiological dynamics prior to its discovery. Although we simulated epidemics across a range of plausible epidemiological dynamics, our models represent a timeframe prior to the ascertainment of COVID-19 cases and sequencing of SARS-CoV-2 genomes and thus prior to when these models could be empirically validated.

Discussion

The genomic diversity of SARS-CoV-2 during the early pandemic presents a paradox. Lineage A viruses are at least two mutations closer to bat coronaviruses, indicating that the ancestor of SARS-CoV-2 arose from this lineage. However, lineage B viruses predominated early in the pandemic, particularly at the Huanan market, indicating that this lineage began spreading earlier in humans. Further complicating this matter is the molecular clock of SARS-CoV-2 in humans, which rejects a single-introduction origin of the pandemic from a lineage A virus. Here, we resolve this paradox by showing that early SARS-CoV-2 genomic diversity and epidemiology is best explained by at least two separate zoonotic transmissions, in which lineage A and B progenitor viruses were both circulating in non-human mammals prior to their introduction into humans (figs. S30 and S31).

The most probable explanation for the introduction of SARS-CoV-2 into humans involves zoonotic jumps from as-yet undetermined, intermediate host animals at the Huanan market (*34*, *38*, *39*). Through late-2019 the Huanan market sold animals that are known to be susceptible to SARS-CoV-2 infection and capable of intra-species transmission (*40–42*). The presence of potential animal reservoirs, coupled with the timing of the lineage B primary case and the geographic clustering of early cases around the Huanan market (*39*), support the hypothesis that SARS-CoV-2 lineage B jumped into humans at the Huanan market in mid-November 2019.

In a related study (*39*), we show that the two earliest lineage A cases are more closely positioned geographically to the Huanan market than expected compared with other COVID-19 cases in Wuhan in early 2020, despite having no known association with the market. This geographic proximity is consistent with a separate and subsequent origin of lineage A at the Huanan market in late-November 2019. The presence of lineage A virus at the Huanan market was confirmed by Gao *et al.* (*43*) from a sample taken from discarded gloves.

The high extinction rate of SARS-CoV-2 transmission chains, observed in both our simulations and real-world data (*44*), indicates that the two zoonotic events establishing lineages A and B may have been accompanied by additional, cryptic introductions. However, such introductions could easily be missed, particularly if their subsequent transmission chains quickly went extinct or the introduced viruses had a lineage A or B haplotype. Failed introductions of intermediate haplotypes are also possible. Critically, we have no evidence of subsequent zoonotic introductions in late-December leading up to the closure of the Huanan market on 1 January 2020. By then, the susceptible host animals that had been documented at the market during the previous months were no longer found in the Huanan market (*34*).

Other coronavirus epidemics and outbreaks in humans, including SARS-CoV-1, MERS-CoV, and, most recently, porcine deltacoronavirus in Haiti, have been the result of repeated introductions from animal hosts (*45–47*). These repeated introductions were easily identifiable because human viruses in these outbreaks were more closely related to viruses sampled in the animal reservoirs than to other human viruses. However, the genomic diversity within the putative SARS-CoV-2 animal reservoir at the Huanan market was likely shallower than that seen in SARS-CoV-1 and MERS-CoV reservoirs (*45*, *46*, *48*). Hence, even though lineages A and B had nearly identical haplotypes, their MRCA likely existed in an animal reservoir. The ability to disentangle repeated introductions of SARS-CoV-2 from a shallow genetic reservoir has previously been shown in the early SARS-CoV-2 epidemic in Washington state, where two viruses, separated by two mutations, were independently introduced from, and shared an MRCA in, China (figs. S23 and S30 and supplementary text) (*11*).

Successful transmission of both lineage A and B viruses after independent zoonotic events indicates that evolutionary adaptation within humans was not needed for SARS-CoV-2 to spread (*49*). We now know that SARS-CoV-2 can readily spread after reverse-zoonosis to Syrian hamsters (*Mesocricetus auratus*), American mink (*Neovison vison*), and white-tailed deer (*Odocoileus virginianus*), indicating its host generalist capacity (*50–55*). Furthermore, once an animal virus acquires the capacity for human infection and transmission,

the only remaining barrier to spillover is contact between humans and the pathogen. Thereafter, a single zoonotic transmission event indicates the conditions necessary for spillovers have been met, which portends additional jumps. For example, there were at least two zoonotic jumps of SARS-CoV-2 into humans from pet hamsters in Hong Kong (*56*) and dozens from minks to humans on Dutch fur farms (*52*, *53*).

We show that it is highly unlikely that SARS-CoV-2 circulated widely in humans earlier than November 2019 and that there was limited cryptic spread, with, at most, dozens of SARS-CoV-2 infections in the weeks leading up to the inferred tMRCA, but likely far fewer. By late-December, when SARS-CoV-2 was identified as the etiological agent of COVID-19 (*8*), the virus had likely been introduced into humans multiple times as a result of persistent contact with a viral reservoir.

Materials and methods summary

Materials and methods described in full detail can be found in the supplementary materials.

Sequence data

We queried the GISAID database (*57*), GenBank, and National Genomics Data Center of the China National Center for Bioinformatics (CNCB), for complete high-coverage SARS-CoV-2 genomes collected by 14 February 2020, resulting in a dataset of 787 taxa belonging to lineages A and B and 20 taxa with C/C or T/T haplotypes. Genomes were aligned using MAFFT v7.453 (*58*) to the SARS-CoV-2 reference genome (Wuhan/Hu-1/2019) and 388 sites were masked at the 5′ and 3′ ends and at sites based on De Maio *et al.* (*59*). All genome accessions are available in data S1 and S2.

Progenitor genome reconstruction and reversion analysis

We reconstructed the progenitor of SARS-CoV-2, the recombinant common ancestor (the recCA). We (i) inferred a maximum likelihood tree of 31 sarbecovirus genomes (SARS-CoV-2 and 30 closely related sarbecoviruses sampled from bats and pangolins) across 15 predefined non-recombinant regions (*13*) with IQ-TREE v2.0.7 (*60*), (ii) inferred the sequence of the ancestor of SARS-CoV-2 in each tree with TreeTime v0.8.1 (*61*), and (iii) concatenated the resulting sequences. We next inferred a maximum likelihood tree of the 787 SARS-CoV-2 taxa with IQ-TREE and performed ancestral state reconstruction with TreeTime to identify substitutions that were reversions from Wuhan-Hu-1 to the recCA across the SARS-CoV-2 phylogeny.

Phylodynamic inference and epidemic simulations

We performed phylodynamic inference using BEAST v1.10.5 (*62*) with the 787-taxa dataset to infer the ancestral haplotype and the tMRCA of SARS-CoV-2 (and the tMRCAs of lineages A and B), employing a non-reversible random-effects substitution model and exploring unconstrained rooting, recCA-rooting, fixing the ancestral haplotype as a root, and outgroup rooting. SARS-CoV-2-like epidemics were simulated with FAVITES-COVID-Lite v0.0.1 (*22*, *63*) using a scale-free network of 5 million individuals and a customized extension of the SAPHIRE model (*64*), producing coalescent trees on which we simulated mutations. We calculated the Bayes factor comparing the support of two introductions of SARS-CoV-2 to one introduction by considering the posterior probabilities of the four most likely ancestral haplotypes from the phylodynamic inference (Lineage A, Lineage B, C/C, and T/T), the frequencies of the phylogenetic structures associated with introductions of these haplotypes in the epidemic simulations, and equal prior probabilities for each ancestral haplotype and one versus two introductions.

We connected the phylodynamic inference and epidemic simulations via a rejection sampling-based approach (*23*), accounting for the tMRCAs of lineages A and B and the earliest documented COVID-19 illness onset and hospitalization dates. We then inferred the timing of the introductions of lineages A and B and the infections and hospitalizations for each lineage. The proportion of epidemic simulations that went extinct (*i.e.*, no onward transmission by the end of the simulation) was used to approximate the number of SARS-CoV-2 introductions needed to result in two introductions with sustained onward transmission.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S31
Tables S1 to S15
References (67–110)
MDAR Reproducibility Checklist
Data S1 to S3

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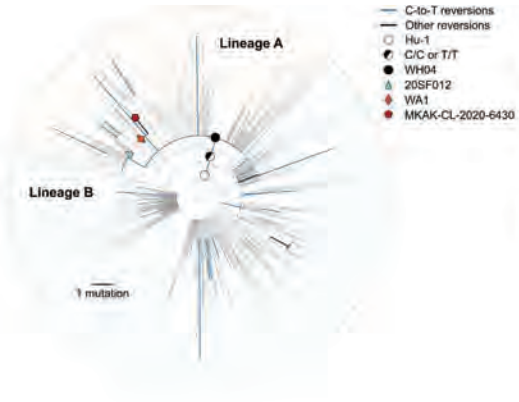

Fig. 1. Maximum likelihood phylogeny of the early SARS-CoV-2 pandemic, showing nucleotide reversions and putative candidates for the ancestral haplotype at the most common recent ancestor (MRCA). Putative ancestral haplotypes are identified with colored shapes. Reversions from the Hu-1 reference genotype to the recCA are colored. Blue represents C-to-T reversions and black indicates all other reversions. The tree is rooted on Hu-1 to show reversion dynamics to the recCA.

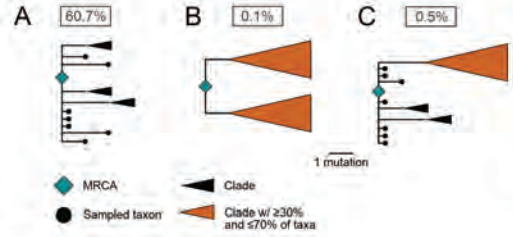

Fig. 2. Probability of phylogenetic structures arising from a single introduction of SARS-CoV-2 in epidemic simulations. (A) A large polytomy of at least 20 descendent lineages, consistent with the base of both lineages A and B. (B) Topology matching a C/C ancestral haplotype: two clades each one mutation from the ancestor, both with polytomies of at least 20 descendent lineages. (C) Topology matching either a lineage A or lineage B ancestral haplotype: a basal polytomy with at least 20 descendent lineages including a large clade separated by two mutations, also possessing a polytomy of at least 20 descendent lineages. Basal taxa have short branch lengths for clarity. The probability of each phylogenetic structure after a single introduction is reported in the box.

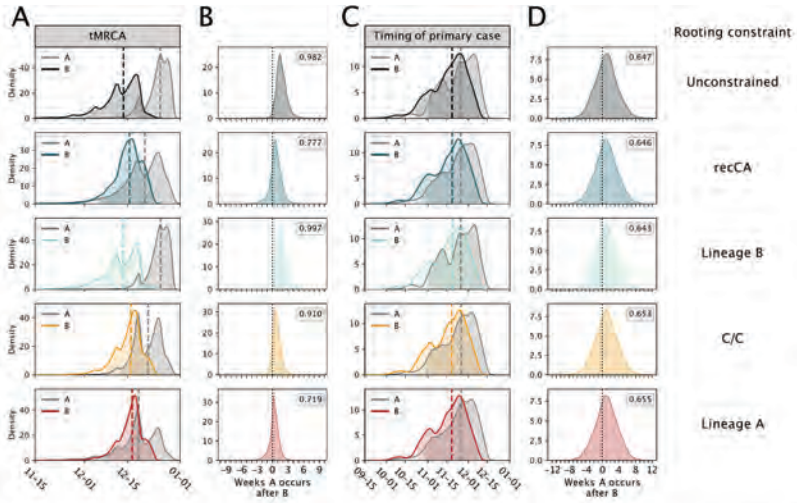

Fig. 3. Comparison of the tMRCA and primary case dates for lineage A and lineage B across rooting strategies. Each row represents a different rooting constraint in phylodynamic analysis. with lineage B, C/C and lineage A representing a fixed ancestral haplotype. (**A**) The tMRCA for lineages A and B. (**B**) The number of weeks the tMRCA of lineage A occurs after the tMRCA of lineage B. (**C**) The timing of the primary case for lineages A and B. (**D**) The number of weeks the time of the primary case of lineage A occurs after the time of the primary case of lineage B. Long dashed lines indicate the median and shading represents the 95% HPD for each distribution. Short dashed lines indicate 0 weeks difference between lineages A and B. Posterior probability that lineage A originated after lineage B is reported in the grey box.

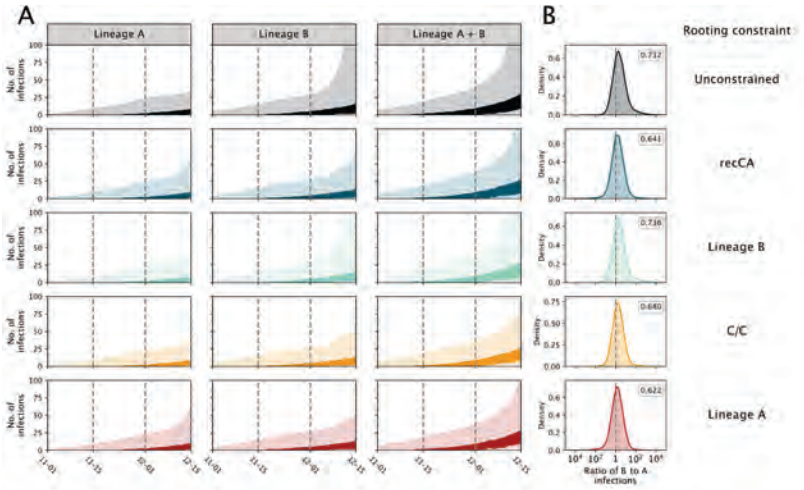

Fig. 4. Dynamics of simulated SARS-CoV-2 epidemics resulting from separate introductions of lineages A and B. Each row represents a different rooting constraint in phylodynamic analysis, with lineage B, C/C and lineage A representing a fixed ancestral haplotype. (**A**) Estimated number of infections. The header of each column indicates whether the number of infections are caused by lineage A, lineage B, or the two lineages combined. Darker and lighter shading represent the 50% and 95% HPD, respectively. (**B**) The log ratio of lineage B to lineage A infections on 15 December 2019. Posterior probability of having more lineage B infections than lineage A reported in the grey box.

Table 1. Posterior probabilities of inferred ancestral haplotype at the MRCA of SARS-CoV-2. Positions 8782 and 28144 are indicated in parentheses. Representative genome is that with its sequence matching the haplotype. "No market" excludes 15 market-associated genomes (13 lineage B genomes associated with the Huanan market plus one lineage A and one lineage B genome not associated with the Huanan market). *BF > 10. **BF > 100. ***BF > 1000; BFs are in favor of hypothesis rejection.

Haplotype	Mutations from Hu-1 reference	Representative genome	Phylodynamic analysis		
			Unconstrained (%)	No market (%)	recCA (%)
B (C/T)	N/A	Hu-1	80.85†	62.96†	8.18
A (T/C)	C8782T+T28144C	WH04	1.68*	5.73*	77.28†
C/C	T28144C	N/A	10.32	23.02	10.49
T/T	C8782T	N/A	0.92*	1.68*	3.71*
A+C29025T (T/C)	C8782T+T28144C+C29095T	20SF012	<0.01***	<0.01***	0.20**
A.1 (T/C)	C8782T+T28144C+C18060T	WA1	<0.01***	<0.01***	0.04**

†Haplotype with greatest posterior probability; reference for BF.

Science

The molecular epidemiology of multiple zoonotic origins of SARS-CoV-2

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RESEARCH ARTICLES

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The Huanan Seafood Wholesale Market in Wuhan was the early epicenter of the COVID-19 pandemic

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Understanding how severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019 is critical to preventing zoonotic outbreaks before they become the next pandemic. The Huanan Seafood Wholesale Market in Wuhan, China, was identified as a likely source of cases in early reports but later this conclusion became controversial. We show the earliest known COVID-19 cases from December 2019, including those without reported direct links, were geographically centered on this market. We report that live SARS-CoV-2 susceptible mammals were sold at the market in late 2019 and, within the market, SARS-CoV-2-positive environmental samples were spatially associated with vendors selling live mammals. While there is insufficient evidence to define upstream events, and exact circumstances remain obscure, our analyses indicate that the emergence of SARS-CoV-2 occurred via the live wildlife trade in China, and show that the Huanan market was the epicenter of the COVID-19 pandemic.

On 31 December 2019, the Chinese government notified the World Health Organization (WHO) of an outbreak of severe pneumonia of unknown etiology in Wuhan, Hubei province (*1–4*), a city of approximately 11 million people. Of the initial 41 people hospitalized with unknown pneumonia by 2 January 2020, 27 (66%) had direct exposure to the Huanan Wholesale Seafood Market (hereafter, "Huanan market") (*2, 5, 6*). These first cases were confirmed to be infected with a novel coronavirus, subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and were suffering from a disease later named coronavirus disease 2019 (COVID-19). The initial diagnoses of COVID-19 were made in several hospitals independently between 18 and 29 December 2019 (*5*). These early reports were free from ascertainment bias as they were based on signs and symptoms before the Huanan market was identified as a shared risk factor (*5*). A subsequent systematic review of all cases notified to China's National Notifiable Disease Reporting System by hospitals in Wuhan as part of the joint WHO-Chinese "WHO-convened global study of origins of SARS-CoV-2: China Part" (hereafter, "WHO mission report") (*7*) showed that 55 of 168 of the earliest known COVID-19 cases were associated with this market. However, the observation that the preponderance of early cases were linked to the Huanan market does not establish that the pandemic originated there.

Sustained live mammal sales during 2019 occurred at the Huanan and three other markets in Wuhan, including wild and farmed wild-life (*8*). Several of these species are known to be experimentally susceptible to SARS-related coronaviruses (SARSr-CoVs), such as SARS-CoV (hereafter, "SARS-CoV-1") and SARS-CoV-2 (*9–11*). During the early stages of the COVID-19 pandemic, animals sold at the Huanan market

were hypothesized to be the source of the unexplained pneumonia cases (*12–19*) (data S1), consistent with the emergence of SARS-CoV-1 from 2002-2004 (*20*), as well as other viral zoonoses (*21–23*). This led to the decision to close and sanitize the Huanan market on 1 January 2020, with environmental samples also being collected from vendors' stalls (*7, 12, 24*) (data S1).

Determining the epicenter of the COVID-19 pandemic at a neighborhood- rather than city-level could help resolve if SARS-CoV-2 had a zoonotic origin, similar to SARS-CoV-1 (*20*). In this study, we obtained data from a range of sources to test the hypothesis that the COVID-19 pandemic began at the Huanan market. Despite limited testing of live wildlife sold at the market, collectively, our results provide evidence that the Huanan market was the early epicenter of the COVID-19 pandemic and suggest that SARS-CoV-2 likely emerged from the live wildlife trade in China. However, events upstream of the market, as well as exact circumstances at the market, remain obscure, highlighting the need for further studies to understand and lower the risk of future pandemics.

Results

Early cases lived near to and centered on the Huanan market

The 2021 WHO mission report identified 174 COVID-19 cases in Hubei province in December 2019 after careful examination of reported case histories (*7*). Although geographical coordinates of the residential locations of the 164 cases who lived within Wuhan were unavailable, we were able to reliably extract the latitude and longitude coordinates of 155 cases from maps in the report (figs. S1 to S8).

While early COVID-19 cases occurred across Wuhan, the majority clustered in central Wuhan near the west bank of the Yangtze River, with a high density of cases near to, and surrounding, the Huanan market (Fig. 1A). We used a kernel density estimate (KDE) to reconstruct an underlying probability density function from which the home locations for each case were drawn (*25*). Using all 155 December 2019 cases, the location of the Huanan market lies within the highest density contour that contains 1% of the probability mass (Fig. 1B). For a KDE estimated using the 120 cases with no known linkage to the market, the market remains within the highest density 1% contour (Fig. 1C). The clustering of COVID-19 cases in December around the Huanan market (Fig. 1, B and C, insets) contrasts with the pattern of widely dispersed cases across Wuhan by early January through mid-February 2020 (Fig. 1, D and E), which we mapped using location data from individuals using a COVID-19 assistance app on Sina Weibo (*26*). Weibo-based data analyses show that, unlike early COVID-19 cases, by January and February many of the sick who sought help resided in highly populated areas of the city, and particularly in areas with a high density of older people (Fig. 1E and figs. S9 and S10).

We also investigated whether the December COVID-19 cases were closer to the market than expected based on an empirical null distribution of Wuhan's population density (data from worldpop.org (*27, 28*)), with its median distance to the Huanan market of 16.11km (*25*). To account for older individuals being more likely to be hospitalized and sick with COVID-19 (*29*), we age-matched the population data to the December 2019 COVID-19 case data. We considered three categories of cases, and they were all significantly closer to the Huanan market than expected: (*i*) all cases (median 4.28km: $p<0.001$), (*ii*) cases linked directly to the Huanan market (median 5.74km; $p<0.001$), and (*iii*) cases with no evidence of a direct link to the Huanan market (median 4.00km: $p<0.001$) (Fig. 2A). The cases with no known link to the market on average resided closer to the market than the cases with links to the market ($p=0.029$). Furthermore, the distances between the center-points (Fig. 2B) and the Huanan market were shorter than expected for all categories of December cases compared with the empirical null distribution of Wuhan's population density (Fig. 2A). For all the December cases the center-point was located 1.02km away ($p=0.007$); the center-point for cases with market links was 2.28km away ($p=0.034$), and the center-point for the cases with no reported link to the market was 0.91km away ($p=0.006$). In comparison, the center-point of age-matched samples drawn from the empirical null distribution was 4.65km away from the market (Fig. 2A).

We tested the robustness of our results to the possibility of ascertainment bias (*25*). For all mapped cases ($n=155$), under the 'center-point distance to the Huanan market' test, the 38 cases residing closest to the market (within a radius of 1.6km) could be removed from the data set before losing significance at the $\alpha=0.05$ level (fig. S12). For the 'median distance to Huanan market' test, we could remove 98 (63%) ($r=5.8$km). For cases not directly linked to the Huanan market (n=120), we could remove 36 (30%) ($r=1.5$km) and 81 (68%) ($r=4.3$km) for the two tests, respectively, before losing significance at the $\alpha=0.05$ level (fig. S12).

We performed a spatial relative risk analysis (*25*) to compare December 2019 COVID-19 cases with January-February 2020 cases, reported via Weibo (Fig. 2C). The Huanan market is located within a well-defined area with high case density that would be expected to be observed in fewer than one in 100,000 samplings of the Weibo data empirical distribution (relative risk analysis in Fig. 2C, control distribution in Fig. 1D). No other regions in Wuhan showed a comparable case density.

Both early lineages of SARS-CoV-2 were geographically associated with the market

Two lineages of SARS-CoV-2 designated A and B (*30*) have co-circulated globally since early in the COVID-19 pandemic (*31*). Until a report in a recent preprint (*24*), only lineage B

sequences had been sampled at the Huanan market. The eleven lineage B cases from December 2019, for which we have location information, resided closer than expected to the Huanan market compared to the age-matched Wuhan population distribution (median 8.30km; p=0.017) (*25*). The center-point of the eleven lineage B cases was 1.95km from the Huanan market, also closer than expected (p=0.026). The two lineage A cases for which we have location information involved the two earliest lineage A genomes known to date. Neither case reported any contact to the Huanan market (*7*). The first case was detected before any knowledge of a possible association of unexplained pneumonia in Wuhan with the Huanan market (*5*) and therefore could not have been a product of ascertainment bias in favor of cases residing near the market. The second had stayed in a hotel near the market (*32*) for the five days preceding symptom onset (*25*). Relative to the age-matched Wuhan population distribution, the first individual resided closer to the Huanan market (2.31km) than expected (p=0.034). While the exact location of the hotel near the market was not reported (*32*), there are at least 20 hotels within 500 m (table S1). Under the conservative assumption that the hotel could have been located as far as 2.31km from the Huanan market (as was the residence of the other lineage A case), and assuming this location is comparable to a residential location given the timing of the stay prior to symptom onset (*25*), it would be unlikely to observe both the earliest lineage A cases this near to the Huanan market (p=0.001 or less). That both identified lineage A cases had a geographical connection to the market, in combination with the detection of lineage A within the market (*24*), support the likelihood that during the early epidemic lineage A was, like lineage B, disseminating outward from the Huanan market into the surrounding neighborhoods.

Our statistical results were robust to a range of factors, for example, the use of an empirical control distribution based on presumptive COVID-19 cases locations later in the Wuhan epidemic (Weibo data); laboratory-confirmed versus clinically-diagnosed cases; and uncertainty in case location or missing data (figs. S13 to S15) (*25*). For instance, we artificially introduced location uncertainty ('noise') in each case location in our data set by randomly re-sampling each point within a circle of radius 1000m centered on its original center-point; the conclusions were unaffected (fig. S13). The extraction method we employed actually introduced up to about 50m of noise in each case location estimate (fig. S7), ruling out the possibility that our overall results were affected by this source of error. The results were also robust when corrected for multiple hypothesis testing (table S4).

Wild animal trading in Wuhan markets

In addition to selling seafood, poultry, and other commodities, the Huanan market was among four markets in Wuhan reported to consistently sell a variety of live, wild-captured or farmed, mammal species in the years and months leading up to the COVID-19 pandemic (*8*). There are, however, no prior reports of which species, if any, were sold at the Huanan market in the months leading up to the pandemic. Here, we report that multiple plausible intermediate wildlife hosts of SARS-CoV-2 progenitor viruses, including red foxes (*Vulpes vulpes*), hog badgers (*Arctonyx albogularis*) and common raccoon dogs (*Nyctereutes procyonoides*), were sold live at the Huanan market up until at least November of 2019 (Table 1 and table S5). No reports are known to be available for SARS-CoV-2 test results from these mammals at the Huanan market. Despite a general slow-down in live animal sales during the winter months, we report that raccoon dogs that are sold for both meat and fur were consistently available for sale throughout the year, including at the Huanan market in November 2019 (Table 1 and table S5).

There were potentially many locations in Wuhan, a city of 11 million, that would have been equally or more likely than the Huanan market to sustain the first recognized cluster of a new respiratory pathogen had its introduction not been linked to a live animal market, including other shopping venues, hospitals, elder care facilities, workplaces, universities, and places of worship. To investigate possible sites, we compared the relative extent of intra-urban human traffic to the Huanan market versus other locations within the city of Wuhan using a location-specific data set of social media check-ins in the Sina Visitor System (*25, 33*). We found at least 70 other markets throughout the city of Wuhan that received more social media visitors than the Huanan market (Fig. 3). To extend this analysis beyond only markets, we also used a subsequently published list of known SARS-CoV-2 superspreader locations (*34*) to identify 430 locations in Wuhan that may have been at high risk for superspreader events and which received more check-ins than the Huanan market (Fig. 3, inset). The Huanan market accounted for 0.12% (120 of 98,146) of social media check-ins to markets in the data set that received at least as many check-ins as the Huanan market. The market accounted for 0.04% (120 of 262,233) of all social media check-ins to the >400 sites in Wuhan identified as especially likely to be potential superspreader locations and which received at least as many social media visits as the Huanan market. Considering the number of check-ins to all four markets selling live, wild animals in Wuhan (combined), they accounted for 0.21% (206 of 98,146) of market visits and 0.079% (206 of 262,233) of visits to the 430 potential superspreader sites, where a new respiratory disease might first be noticed in a large city.

A data set from the Chinese Center for Disease Prevention and Control (CCDC) report dated 22 January 2020 (data S1) (*12, 13, 15, 16*) was made publicly available in June 2020 (*24, 35*). 585 environmental samples were initially taken from various surfaces in the Huanan market on 1 and 12 January 2020 by the

CCDC (tables S6 and S7 and data S1) (*12, 13, 15, 16, 24, 35*), with further samples taken through the market during January and February (*24*). We extended the analysis in the WHO mission report (*7*) by integrating public online maps and photographic evidence, data from public business registries (table S8 and data S2), information about which live mammal species were sold at the Huanan market in late 2019 (Table 1 and table S5), and the CCDC report (data S1). We reconstructed the floor plan of the market and integrated information from business registries of vendors at the market (fig. S16 and table S8), as well as an official report (*36*) recording fines to three business owners for illegal sale of live mammals (data S2) (*36*). From this, we identified an additional five stalls that were likely selling live or freshly butchered mammals or other unspecified meat products in the southwest corner of the western section of the market (Fig. 4A, figs. S16 and S17, and table S6).

Five of the SARS-CoV-2-positive environmental samples were taken from a single stall selling live mammals in late 2019 (table S6). Further, the objects sampled showed an association with animal sales, including a metal cage, two carts (of the kind frequently used to transport mobile animal cages) and a hair/feather remover (table S6). No human COVID-19 cases were reported there (*7, 12*). The same stall was visited by one of us (ECH) in 2014, who then observed live raccoon dogs housed in a metal cage stacked on top of a cage with live birds (Fig. 4A) (*37*). A recent report (*24*) identified that the grates outside of this stall, upon which animal cages were stacked (*37*), were positive for SARS-CoV-2.

Positive environmental samples linked both to live mammal sales and to human cases at the Huanan market

We used a spatial relative risk analysis to identify potential regions of the market with an increased density of positive environmental samples (*25*). We found evidence ($p<0.05$) of a region in the southwest area of the market where live mammals were on sale (Fig. 4B). Although environmental sampling of the market was incomplete and spatially heterogeneous (data S1 and table S6), our analysis accounts for the empirical environmental sampling distribution, which was biased toward 'stalls related to December cases' as well as 'stalls that sold livestock, poultry, farmed wildlife' (*7*) (Fig. 4, C and D). The 'distance to the nearest vendor selling live mammals' and 'distance to the nearest human case' were independently predictive of environmental sample positivity ($p=0.004$ and 0.014, respectively for N=6; table S9). To further investigate the robustness of these findings to possible sampling biases, we considered three scenarios: (*i*) oversampling of live mammal and unknown meat stalls, (*ii*) overcounting of positive samples, and (*iii*) exclusion of the seafood stand near the wildlife area of the market (with five positive samples) from our analysis (table S10). In each case, the distance to live mammal vendors remained predictive of environmental sample positivity, and the region of increased positive sample density in the southwest corner of the western section of the market remained consistent (fig. S18).

Finally, to analyze the spatial patterning of human cases within the Huanan market, we plotted cases as a function of symptom onset from the WHO mission report (*7*) (Fig. 5A and table S11) (*25*). All eight COVID-19 cases detected prior to 20 December were from the western side of the market, where mammal species were also sold (Fig. 5, B and C). Unlike SARS-CoV-2 positive environmental samples (Fig. 4, A and C), we found that COVID-19 cases were more diffuse throughout the building (Fig. 5).

Study limitations

There are several limitations to our study. We have been able to recover location data for most of the December-onset COVID-19 cases identified by the WHO mission (*7*) and have been able to do so with sufficient precision to support our conclusions. However, we do not have access to the precise latitude and longitude coordinates of all these cases. Should such data exist, they may be accompanied by additional metadata, some of which we have reconstructed, but some of which, including the date of onset of each case, would be valuable for ongoing studies. We also lack direct evidence of an intermediate animal infected with a SARS-CoV-2 progenitor virus either at the Huanan market or at a location connected to its supply chain, like a farm. Additionally, no line list of early COVID-19 cases is available and we do not have complete details of environmental sampling, though compared to many other outbreaks, we have more comprehensive information on early cases, hospitalizations and environmental sampling (*7*).

Discussion

Several lines of evidence support the hypothesis that the Huanan market was the epicenter of the COVID-19 pandemic and that SARS-CoV-2 emerged from activities associated with live wildlife trade. Spatial analyses within the market show that SARS-CoV-2-positive environmental samples, including cages, carts, and freezers, were associated with activities concentrated in the southwest corner of the market. This is the same section where vendors were selling live mammals, including raccoon dogs, hog badgers, and red foxes, immediately prior to the COVID-19 pandemic. Multiple positive samples were taken from one stall known to have sold live mammals, and the water drain proximal to this stall, as well as other sewerages and a nearby wildlife stall on the southwest side of the market, tested positive for SARS-CoV-2 (*24*). These findings suggest that infected animals were present at the Huanan market at the beginning of the COVID-19

pandemic; however, we do not have access to any live animal samples from relevant species. Additional information, including sequencing data and detailed sampling strategy, would be invaluable to test this hypothesis comprehensively.

In a related study, we infer separate introductions of SARS-CoV-2 lineages A and B into humans from likely infected animals at the Huanan market (*38*). We estimate the first COVID-19 case to have occurred in November 2019, with few human cases and hospitalizations occurring through mid-December (*38*). A recent preprint (*24*) confirms the authenticity of the CCDC report (data S1) and records additional positive environmental samples in the southwestern area of the market selling live animals. This report also documents the early presence of the A lineage of SARS-CoV-2 in a Huanan market environmental sample. This, along with the lineage A cases we report in close geographical proximity to the market in December, challenges the suggestion that the market was simply a superspreading event, which would be lineage-specific. Rather, it adds to the evidence presented here that lineage A, like lineage B, may have originated at the Huanan market then spread from this epicenter into the neighborhoods surrounding the market and then beyond.

Several observations suggest that the geographic association of early COVID-19 cases with the Huanan market is unlikely to have been the result of ascertainment bias (supplementary text and tables S2 and S3) (*39*). These include: (*i*) few, if any, cases among Huanan market-unlinked individuals are likely to have been detected by active searching in the neighborhoods around the market – only in hospitals – since all cases analyzed here were hospitalized (*7*), (*ii*) public health officials simultaneously became aware of Huanan-linked cases near and far from the Huanan market, not just ones near it (fig. S11) (*5*), (*iii*) Huanan-unlinked cases would not be expected to live significantly closer to the market than linked cases if they had been ascertained as contacts traced from those market-linked cases, and (*iv*) seroprevalence in Wuhan was highest in the districts around the market (*40*, *41*). It is also noteworthy that the December 2019 COVID-19 cases we consider here were identified based on reviews of clinical signs and symptoms, not epidemiological factors such as where they resided or links to the Huanan market (*7*) and that excess deaths from pneumonia rose first in the districts surrounding the market (*42*). Moreover, the spatial relationship with the Huanan market remains after removing the two-thirds of the unlinked cases residing nearest the market.

One of the key findings of our study is that 'unlinked' early COVID-19 patients, those who neither worked at the market or knew someone who did, nor had recently visited the market, resided significantly closer to the market than patients with a direct link to the market. The observation that a substantial proportion of early cases had no known epidemiological link had previously been used as an argument against a Huanan market epicenter of the pandemic. However, this group of cases resided significantly closer to the market than those who worked there, indicating that they had been exposed to the virus at, or near, the Huanan market. For market workers, the exposure risk was their place of work not their residential locations, which were significantly further afield than those cases not formally linked to the market.

Our spatial analyses show how patterns of COVID-19 cases shifted between late 2019, when the outbreak began (*43*), and early 2020, as the epidemic spread widely across Wuhan. COVID-19 cases in December 2019 were associated with the Huanan market in a manner unrelated to Wuhan population density or demographic patterns, unlike the wide spatial distribution of cases observed during later stages of the epidemic in January and February. This observation fits with the evidence from other sources that SARS-CoV-2 was not widespread in Wuhan at the end of 2019. For example, no SARS-CoV-2-positive sera or influenza-like illness (ILI) reports were recorded among more 40,000 blood donor samples collected up to December 2019 (*44*, *45*), and none of thousands of samples taken from ILI patients at Wuhan hospitals in October-December 2019 tested for SARS-CoV-2 RNA was positive (*7*).

The sustained presence of a potential source of virus transmission into the human population in late 2019, plausibly from infected live mammals sold at the Huanan market, offers an explanation of our findings and the origins of SARS-CoV-2. The pattern of COVID-19 cases reported for the Huanan market, with the earliest cases in the same part of the market as the wildlife sales and evidence of at least two introductions (*38*), resembles the multiple cross-species transmissions of SARS-CoV-2 subsequently observed during the pandemic from animals to humans on mink farms (*46*), and from infected hamsters to humans in the pet trade (*47*). There was an extensive network of wildlife farms in western Hubei province, including hundreds of thousands of raccoon dogs on farms in Enshi prefecture, which supplied the Huanan market (*48*). This region of Hubei contains extensive cave complexes housing *Rhinolophus* bats, which carry SARSr-CoVs (*49*). SARS-CoV-1 was recovered from farmed masked palm civets from Hubei in 2003 and 2004 (*20*). The animals on these farms (nearly 1 million) were rapidly released, sold, or killed in early 2020 (*48*), apparently without testing for SARS-CoV-2 (*7*). Live animals sold at the market (Table 1) were apparently not sampled either. By contrast, during the SARS-CoV-1 outbreaks farms and markets remained open for over a year after the first human cases occurred, allowing sampling of viruses from infected animals (*20*).

The live animal trade and live animal markets are a common theme in virus spillover events (*21–23*, *50*), with markets such as the Huanan market selling live mammals being in the highest risk category (*51*). The events leading up to the

COVID-19 pandemic mirror the SARS-CoV-1 outbreaks from 2002-2004, which were traced to infected animals in Guangdong, Jiangxi, Henan, Hunan, and Hubei provinces in China (*20*). Maximum effort must now be applied to elucidate the upstream events that might have brought SARS-CoV-2 into the Huanan market, culminating in the COVID-19 pandemic. To reduce the risk of future pandemics we must understand, and then limit, the routes and opportunities for virus spillover.

Methods summary

Ethics statement

This research was reviewed by the Human Subject Protection Program at the University of Arizona and the Institutional Review Board at The Scripps Research Institute and determined to be exempt from IRB approval because it constitutes secondary research for which consent is not required.

Data sources

COVID-19 case data from December 2019 was obtained from the WHO mission report (*7*) and our previous analyses (*5*). Location information was extracted and sensitivity analyses performed to confirm accuracy and assess potential ascertainment bias. Geotagged January/February 2020 data from Weibo COVID-19 help seekers was obtained from the authors (*26*). Population density data was obtained from worldpop.org (*27*). Sequencing- or qPCR-based environmental sample SARS-CoV-2 positivity from the Huanan market was obtained from a January 2020 China CDC report (data S1) (*24*).

Wildlife trading at the Huanan market

Animal sales from Wuhan wet markets immediately prior to the COVID-19 pandemic was previously reported (*8*) and in this study we report details about animals for sale at the Huanan market up until November 2019.

Spatial analyses of COVID-19 cases

Haversine distances to the Huanan market were calculated for each of the geolocated December 2019 cases. Centerpoints and median distances from cases to the Huanan market were calculated separately for (1) all 155 cases, (2) the 35 cases epidemiologically linked to the Huanan market, (3) for the 120 cases not epidemiologically linked to the market, (4) the eleven lineage B cases, and (5) the earliest lineage A case. These distances were also calculated for the 737 Weibo help seekers from 8 January to 10 February 2020 (*26*). Empirical null distributions were generated from the population density data and the Weibo data. The population density null distributions were age-matched to the December 2019 cases.. Kernel density estimates were also generated for the market-linked cases, unlinked-cases and all cases, to infer a probability density function from which the cases could have been drawn. Highest-density contours representing specific probability masses (0.5, 0.25, 0.1, 0.05, and 0.01) were inferred and the location of the market compared to these.

Mobility analyses

To estimate the relative amount of intra-urban human traffic to the Huanan market compared to other locations within the city of Wuhan, we utilized a location-specific dataset of social media check-ins in the Sina Visitor System as shared by Li *et al.* 2015 (*33*). This dataset is based on 1,491,499 individual check-in events across the city of Wuhan from the years 2013-2014 (5-6 years before the start of the COVID-19 pandemic), and 770,521 visits are associated with 312,190 unique user identifiers. Location names and categories were translated using a Python API for Google Translate.

Spatial analyses of environmental samples at the Huanan market

We used the official maps from the China CDC (*12*) (data S1) and WHO map (*7*), as well as satellite photographs (Google Maps, Google Earth, Baidu Maps), aerial photographs, and images of the market in the public domain to reconstruct the floorplan of the market. Market stalls were assigned by categories of the types of goods sold using official reports and data from the TianYanCha.com business directory (table S8). Final maps of the Huanan market were converted into geojson format for spatial analyses. Significance testing of live animal vendors and/or human SARS-CoV-2 cases on the number of positive environmental samples was performed using a binomial GLM. Distances between businesses were defined as the distance between their respective centerpoints and spatial relative risk analysis was performed using the 'sparr' package in R, using linear boundary kernels for edge correction (*52*), with bandwidth selection performed using least squares cross-validation.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
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Tables S1 to S12
References (*54*–*61*)
Data S1 and S2
MDAR Reproducibility Checklist

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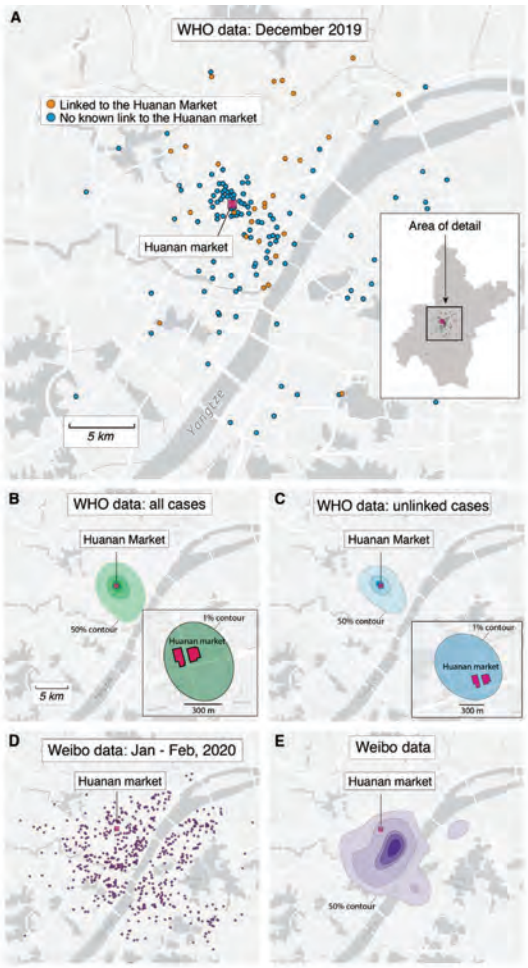

Fig. 1. Spatial patterns of COVID-19 cases in Wuhan in December 2019 and January-February 2020. (A) Locations of the 155 cases we extracted from the WHO mission report (7). Inset: map of Wuhan with December 2019 case indicated with gray dots. (No cases are obscured by the inset.) In both the inset and the main panel the location of the Huanan market is indicated with a red square. (B) Probability density contours reconstructed by a kernel density estimate (KDE) using all 155 COVID-19 cases locations from December 2019. The highest density 50% contour marked is the area for which cases drawn from the probability distribution are as likely to lie inside as outside. Also shown are the highest density 25%, 10%, 5%, and 1% contours. Inset showing an expanded view and the highest density 1% probability density contour. (C) Probability density contours reconstructed using the 120 COVID-19 cases locations from December 2019 that were unlinked to the Huanan market. (D) Locations of 737 COVID-19 cases from Weibo data dating to January and February of 2020. (E) The same highest probability density contours (50% through 1%) for 737 COVID-19 case locations from Weibo data.

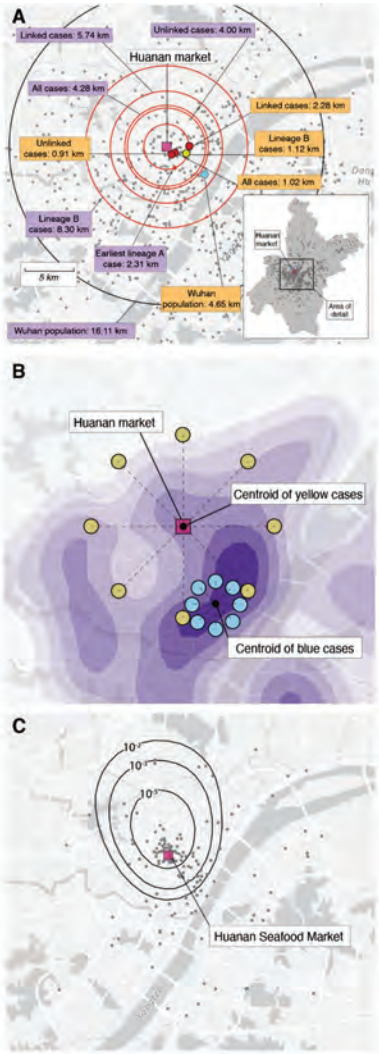

Fig. 2. **Spatial analyses.** (**A**) Inset: map of Wuhan, with gray dots indicating 1000 random samples from worldpop.com null distribution. Main panel: median distance between Huanan market and (1) worldpop.org null distribution shown with a black circle and (2) December cases shown by red circles (distance to Huanan market depicted in purple boxes). Center-point of Wuhan population density data shown by blue dot. Center-points of December case locations shown by red dots ('all', 'linked' and 'unlinked' cases); dark blue dot (lineage A cases); and yellow dot (lineage B cases). Distance from center-points to Huanan market depicted in orange boxes. (**B**) Schematic showing how cases can be near to, but not centered on, a specific location. We hypothesized that if the Huanan market epicenter of the pandemic then early cases should fall not just unexpectedly near to it but should also be unexpectedly centered on it (see Methods). The blue cases show how cases quite near the Huanan market could nevertheless not be centered on it. (**C**) Tolerance contours based on relative risk of COVID-19 cases in December, 2019 versus data from January-February 2020. The dots show the December case locations. The contours represent the probability of observing that density of December cases within the bounds of the given contour if the December cases had been drawn from the same spatial distribution as the January-February data.

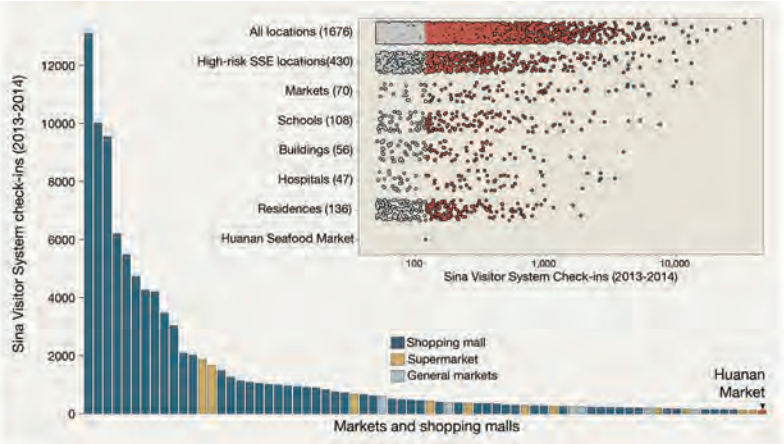

Fig. 3. Visitors to locations throughout Wuhan. Number of social media check-ins in the Sina Visitor System from 2013-2014 as shared by (*33*). Number of visitors to individual markets throughout the city are shown in comparison to the Huanan market. Inset: the total number of check-ins to all individual locations across the city of Wuhan, grouped by category. Locations with more than 50 visitor check-ins are shown, and the locations which received more check-ins than the Huanan market in the same period are shown in red.

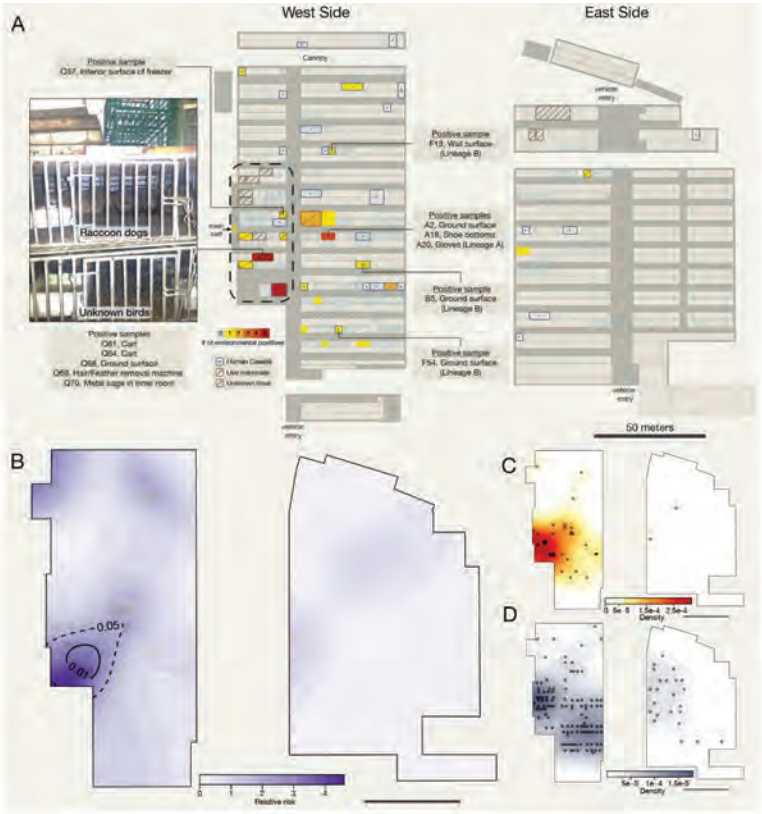

Fig. 4. Map of the Huanan Wholesale Seafood Market. (A) Aggregated environmental sampling and human case data from Huanan Market. Captions (left) describe the types of SARS-CoV-2 positive environmental samples obtained from known live animal vendors and (center) from stalls with samples with known virus lineage. Lineage is unknown unless noted; sequencing data has not been released for some samples and many samples were PCR-positive but not sequenced. Image (left) of raccoon dogs in a metal cage, on top of caged birds, taken in business with five positive environmental samples (photo credit: E.C.H.). Rectangle with dashed outline is used to denote the 'wildlife' section of the market. (B) Relative risk analysis of positive environmental samples. Tolerance contours enclose regions with statistically significant elevation in density of positive environmental samples relative to the distribution of sampled stalls. (C) Distribution of positive environmental samples. Sample locations (centroid of corresponding business) and quantity are shown as black circles. (D) Control distribution for relative risk analysis. All businesses investigated with environmental sampling are shown as black circles (one per business, whether or not a positive sample was found). See table S12 for details on stalls that were SARS-CoV-2-negative.

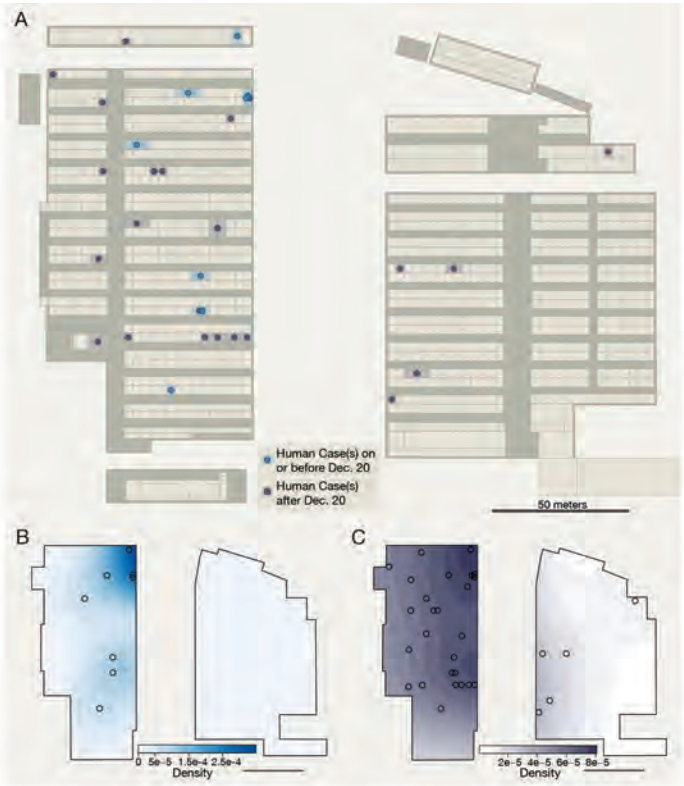

Fig. 5. Location and timing of human cases in Huanan market. (**A**) Outline colors correspond to the timing of the first known case in each business. Individual case timing is denoted by marker color and shown within the outlined business. (**B**) Distribution of known cases on or before December 20th, 2019. Locations of each case are shown as a black circle. (**C**) Distribution of all known human cases in Huanan Market. See table S11 for details on SARS-CoV-2 positive human cases with the Huanan market.

Table 1. Live mammals traded at the Huanan market in November and December 2019

Species (susceptibility*)	Family (susceptibility*)	Order (susceptibility*)	Observed at Huanan market, November 2019
Raccoon dog (*Nyctereutes procyonoides*) (Y)	Canidae (Y)	Carnivora (Y)	Y
Amur hedgehog (*Erinaceus amurensis*)	Erinaceidae	Eulipotyphla	Y
Hog badger (*Arctonyx albogularis*) (Y)	Mustelidae (Y)	Carnivora (Y)	Y
Asian badger (*Meles leucurus*)	Mustelidae (Y)	Carnivora (Y)	Y
Chinese hare (*Lepus sinensis*)	Leporidae (Y)	Lagomorpha (Y)	Y
Chinese bamboo rat (*Rhizomys sinensis*) (Y)	Spalacidae (Y)	Rodentia (Y)	Y
Malayan porcupine (*Hystrix brachyura*)	Hystricidae	Rodentia (Y)	Y
Chinese muntjac (*Muntiacus reevesi*)	Cervidae (Y)	Artiodactyla (Y)	Y
Marmot (*Marmota himalayana*)	Sciuridae	Rodentia (Y)	Y
Red fox (*Vulpes vulpes*) (Y)	Canidae (Y)	Carnivora (Y)	Y
Siberian weasel (*Mustela sibirica*)	Mustelidae (Y)	Carnivora (Y)	N†
Pallas's squirrel (*Callosciurus erythraeus*)	Sciuridae	Rodentia (Y)	N
Masked palm civet (*Paguma larvata*) (Y)	Viverridae (Y)	Carnivora (Y)	N
Coypu (*Myocastor coypus*)	Echimyidae	Rodentia (Y)	N
Mink (*Neovison vison*) (Y)	Mustelidae (Y)	Carnivora (Y)	N
Red squirrel (*Sciurus vulgaris*)	Sciuridae	Rodentia (Y)	N
Wild boar (*Sus scrofa*) (Y)	Suidae (Y)	Artiodactyla (Y)	N
Complex-toothed flying squirrel (*Trogopterus xanthipes*)	Sciuridae	Rodentia (Y)	N

*Based on live susceptibility findings, serological findings, or ACE2-binding assays. See table S5 for details and associated references.
†Animals listed as "No" were, however, present at Wuhan markets during the 2017–2019 study period (*8*).

Science

The Huanan Seafood Wholesale Market in Wuhan was the early epicenter of the COVID-19 pandemic

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October 20, 2021

Statement on Misinformation about SARS-CoV-2 Origins

To date, the origin of the SARS-CoV-2 virus that caused the COVID-19 pandemic has not been identified, despite intensive efforts to do so. This is not unusual — confirming with 100% certainty the origin of a virus is a long and complicated process. It took 14 years for scientists to find a single bat population that contained all the necessary genetic components of SARS-CoV, the virus that caused the 2003 SARS epidemic. We still do not know the origins of the 2014 Ebola outbreak.

Unfortunately, in the absence of a definitive answer, misinformation and disinformation are filling the void, which does more harm than good. NIH wants to set the record straight on NIH-supported research to understand naturally occurring bat coronaviruses at the Wuhan Institute of Virology, funded through a subaward from NIH grantee EcoHealth Alliance. Analysis of published genomic data and other documents from the grantee demonstrate that the naturally occurring bat coronaviruses studied under the NIH grant are genetically far distant from SARS-CoV-2 and could not possibly have caused the COVID-19 pandemic. Any claims to the contrary are demonstrably false.

The scientific evidence to date indicates that the virus is likely the result of viral evolution in nature, potentially jumping directly to humans or through an unidentified intermediary animal host. Historically, many viruses have emerged from animals to cause epidemics and pandemics, including influenza, Ebola, Zika, West Nile fever, SARS, and more. Importantly, after an intensive investigation, agencies in the U.S Intelligence Community agreed that the virus was not developed as a biological weapon and most agencies assessed that SARS-CoV-2 most likely was not genetically engineered.

Public health and scientific organizations, including NIH, are intensely interested in getting a definitive answer to inform efforts to prevent future events. This effort would benefit from less speculation and more scientific cooperation, especially from China, without which the SARS-CoV-2 origins will be impossible to identify.

Francis S. Collins, M.D., Ph.D.
Director, National Institutes of Health


A Report of the National Science Advisory Board for Biosecurity
RECOMMENDATIONS FOR THE EVALUATION AND OVERSIGHT OF PROPOSED GAIN-OF-FUNCTION RESEARCH
May 2016

Contents

List of Acronyms

Bioethics Commission	Presidential Commission for the Study of Bioethical Issues
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSAT	biological select agents and toxins
BSL	biosafety level
CDC	Centers for Disease Control and Prevention
CoV	coronavirus
DURC	dual use research of concern
FBI	Federal Bureau of Investigation
FDA	Food and Drug Administration
FESAP	Federal Experts Security Advisory Panel
FSAP	Federal Select Agent Program
FTAC-SAR	Fast Track Action Committee on Select Agent Regulations
GOF	gain-of-function
GOFROC	gain-of-function research of concern
HHS	Department of Health and Human Services
HHS Framework	Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets
HPAI	highly pathogenic avian influenza
IBC	Institutional Biosafety Committee
LPAI	low pathogenic avian influenza
MCM	medical countermeasures
MERS	Middle East Respiratory Syndrome
National Academies	National Academies of Sciences, Engineering, and Medicine
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH Guidelines	NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
NSABB	National Science Advisory Board for Biosecurity
RAC	Recombinant DNA Advisory Committee
RBA	risk and benefit assessments
SARS	Severe Acute Respiratory Syndrome
USDA	U.S. Department of Agriculture
USG	U.S. Government

Executive Summary

Research involving pathogens is essential to global health and security. Such research provides insight into the fundamental nature of human-pathogen interactions, enables the assessment of the pandemic potential of emerging infectious agents, and informs public health and preparedness efforts, including the development of medical countermeasures. Several federal policies are in place to help ensure that pathogen research is conducted safely and in ways that minimize the risks of laboratory accidents and security risks. A number of biosafety incidents at Federal facilities in 2014 prompted renewed efforts to promote and enhance biosafety and biosecurity. Concerns were also raised about certain "gain-of-function" (GOF) studies with the potential to generate pathogens with pandemic potential. The concerns centered on whether a pathogen with enhanced transmissibility and/or virulence could be accidentally or intentionally released from a laboratory, potentially exposing surrounding populations and possibly causing a wider pandemic.

The U.S. government (USG), as part of its continued focus on biosafety and biosecurity, undertook a deliberative process to carefully examine the risks and benefits associated with certain GOF studies. The deliberative process involved the National Science Advisory Board for Biosecurity (NSABB), which was tasked with making recommendations to the USG on this topic, and the National Academies of Sciences, Engineering, and Medicine (National Academies), which was tasked to convene two public symposia to generate broad discussion on relevant issues. To further inform NSABB deliberations, the National Institutes of Health (NIH) commissioned two studies – an independent assessment of the risks and benefits associated with GOF studies, conducted by Gryphon Scientific, and an ethical analysis of the issues related to funding and conducting such studies, performed by Professor Michael Selgelid.

The NSABB was charged with advising on the design of the risk and benefit assessments (RBA) for GOF studies and with providing recommendations to the USG on a conceptual approach for evaluating proposed GOF studies. In May 2015 the NSABB issued its *Framework for Guiding the Conduct of Risk and Benefit Assessments of Gain-of-Function Research*, which guided NIH in overseeing the contractor conducting the RBA. In May 2016, informed by the results of the RBA as well as its analysis of the current policy landscape, consideration of relevant ethical issues, and consultations with domestic and international stakeholders, the NSABB finalized the report that follows – *Recommendations for the Evaluation and Oversight of Proposed Gain-of-Function Research* – which describes the Board's analyses and findings, and articulates its recommendations to the USG for the evaluation and oversight of proposed GOF studies.

NSABB Findings:

Finding 1. There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Finding 2. The U.S. government has several policies in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Finding 3. Oversight policies vary in scope and applicability, and do not cover all potential GOFROC, therefore, current oversight is not sufficient for all GOF research of concern.

Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and that the benefits of the research are being fully realized.

Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted because the potential risks associated with the study are not justified by the potential benefits. Decisions about whether specific GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important and need to be taken into account.

Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires both federal and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Finding 7. Funding and conducting GOF research of concern encompasses many issues that are international in nature.

NSABB Recommendations to the U.S. government:

Recommendation 1. Research proposals involving GOF research of concern entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the federal and institutional levels.

As part of this recommendation, the NSABB has proposed a conceptual approach for guiding funding decisions about GOFROC. First, the NSABB identified the attributes of GOFROC, which is research that could generate a pathogen that is: 1) highly transmissible and likely capable of wide and uncontrollable spread in human populations; and 2) highly virulent and likely to cause significant morbidity and/or mortality in humans. Next, the NSABB identified a set of principles that should guide funding decisions for GOFROC. Only research that is determined to be in line with these principles should be funded. Additional risk mitigation measures may be required for certain research studies to be deemed acceptable for funding.

Recommendation 2. An advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOF research of concern.

Recommendation 3. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOF research of concern.

> **Recommendation 3.1.** The U.S. government should develop a system to collect and analyze data about laboratory safety incidents, near-misses, and security breaches as well as the effectiveness of mitigation measures to inform GOF research of concern policy development over time.
>
> **Recommendation 3.2.** The U.S. government should develop or facilitate the development of a system to collect and analyze data about Institutional Review Entity (IRE) challenges, decisions, and lessons learned to provide feedback to the IRE community and to inform policy development for GOF research of concern over time.

Recommendation 4. In general, oversight mechanisms for GOF research of concern should be incorporated into existing policy frameworks when possible.

Recommendation 5. The U.S. government should consider ways to ensure that all GOF research of concern conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOF research of concern.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOF research of concern.

1. Introduction

A robust life sciences research enterprise is necessary to counter the continually evolving threats to public health and national security posed by endemic and emerging pathogens, as well as malicious biological threats. By helping to define the nature of human-pathogen interactions, life sciences research promotes public health and national security not only by enhancing our understanding of pathogen biology and disease pathogenesis, but also by informing biosurveillance and medical countermeasure development. Such research can also aid in the assessment of the pandemic potential of emerging infectious agents, thereby underpinning health policy decisions and preparedness and response efforts.

While the ultimate goal of life sciences research involving pathogens is the protection and promotion of public health, there are inherent associated biosafety and biosecurity risks. Potential risks might arise from laboratory accidents or security breaches that result in laboratory acquired infections or the accidental or deliberate release of a pathogen from containment. Life sciences research also has "dual use" potential. That is, legitimate research may generate information, products, or technologies that could be misused to threaten public health or national security. To mitigate such dual use concerns, as well as potential biosafety and biosecurity risks, research involving pathogens is subject to multiple layers of federal and institutional oversight.

The Gain-of-Function Debate and the U.S. Government Response

Experimental techniques and approaches that modify microorganisms are routinely employed in pathogen research to ascertain the roles of genes and their functional products. Such studies are fundamental to the field of microbiology and facilitate correlation of genetic and phenotypic characteristics – a critical step in deciphering the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis. Such manipulations can result in either diminished (loss-of-function) or enhanced (gain-of-function) biological phenotypes (see Box 1).

Studies that result in the generation of pathogens with enhanced, or gain-of-function (GOF), phenotypes are conducted for a number of valid scientific purposes. Such studies provide information that adds to the scientific knowledge base and can inform biosurveillance, medical countermeasure development, and public policy decision-making related to public health and preparedness. The vast majority of such GOF studies do not raise significant safety or security concerns. However, certain GOF studies involving pathogens have raised concerns about whether a laboratory-generated pathogen with pandemic potential could be accidentally or intentionally released, resulting in significant consequences to public, or perhaps, global health. Concerns have also been raised about whether certain GOF studies could generate information that could enable individuals with malevolent intent to generate a pathogen with pandemic potential.

The controversy over certain GOF studies arose after two groups demonstrated that highly pathogenic avian influenza H5N1 viruses with a small number of experimentally-induced mutations became transmissible between mammals by respiratory droplets.[1,2] In 2012, in response to the controversy associated with publication of the manuscripts describing these findings, the influenza community initiated a voluntary suspension of certain GOF studies involving highly pathogenic avian influenza H5N1 viruses. During that time, policymakers considered whether certain GOF studies should be conducted using federal funds, and if so, how those studies could be safely conducted. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) issued new biosafety guidelines for working with highly pathogenic avian influenza strains.[3,4] The U.S. Department of Health and Human Services (HHS) developed a framework for guiding its funding decisions about GOF projects that may generate H5N1 or H7N9 avian influenza viruses that are transmissible between mammals by respiratory droplets.[5,6]

Concerns regarding laboratory safety and biosecurity were renewed following a number of biosafety incidents at U.S. Federal laboratories reported during the summer of 2014. The incidents did not involve GOF studies *per se* but raised broader concerns about laboratory safety and security as it applies to pathogen research.

Box 1. Gain-of-Function Research

Recently, the phrase "gain-of-function research" has become synonymous with certain studies that enhance the ability of pathogens to cause disease. However, gain-of-function studies, as well as loss-of-function studies, are common in molecular microbiology and are essential to understanding molecular pathogenesis of infectious diseases. Changes to the genome of an organism, whether naturally occurring or directed through experimental manipulations in the laboratory, can result in altered phenotypes, as biological functions are lost or gained. Investigators routinely conduct loss- and gain-of-function experiments to understand the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis.

The term "gain-of-function" is generally used to refer to changes resulting in the acquisition of new, or an enhancement of existing, biological phenotypes. This report further defines "gain-of-function research of concern" to describe the subset of studies that have been the subject of recent debate and have raised potential biosafety and biosecurity implications. These are gain-of-function studies with the potential to generate pathogens with pandemic potential in humans by exhibiting high transmissibility and high virulence. See Section 6 for a more rigorous description of GOF research of concern (GOFROC).

---

[1] Imai, M., et al. *Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets*. Nature 486, 21 June 2012.

[2] Herfst, S., et al. *Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets. Science* 336, 22 June 2012.

[3] Gangadharan, D., Smith, J., and Weyant, R., *Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage*. Morbidity and Mortality Weekly Report 62(RR06); 1-7. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6206a1.htm

[4] *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

[5] *Framework for Guiding Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets*. February 21, 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

[6] Jaffe, H.W., et al. *Avian flu: Extra Oversight for H7N9 Experiments*. Nature 500, 8 August 2013. http://www.nature.com/nature/journal/v500/n7461/full/500151a.html

As one component of comprehensive efforts to review and enhance laboratory biosafety and biosecurity, the U.S. government (USG) embarked on a deliberative process to re-evaluate the risks and benefits of certain GOF research with a goal of developing policy governing the funding and conduct of such research.[7] The deliberative process involved the National Science Advisory Board for Biosecurity (NSABB), which served as the official federal advisory body for providing advice in this area, and the National Academies of Sciences, Engineering, and Medicine (National Academies), which fostered broader scientific and public discussions on the topics. To inform NSABB deliberations, NIH commissioned formal risk and benefit assessments (RBA) of GOF research involving pathogens with pandemic potential and a separate analysis of ethical issues surrounding the conduct of such studies. Stakeholder input was also essential to the process.

The deliberative process was accompanied by a pause in the provision of new federal funds for certain GOF research involving influenza, Middle East Respiratory Syndrome (MERS) or Severe Acute Respiratory Syndrome (SARS) viruses—pathogens determined to have pandemic potential. Specifically:

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. This restriction would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity.*[8]

In parallel, the USG encouraged the research community (both those who receive USG funding and those who do not) to join in adopting a voluntary pause on any ongoing research that involves the types of studies that are subject to the funding restriction above.

NSABB recommendations conveyed in this report will inform the USG as it develops policy about whether certain types of GOF studies on pathogens with pandemic potential should be supported and, if so, how such research proposals should be evaluated to inform funding and oversight decisions. It is expected that the temporary funding pause will be lifted and/or replaced by a decision or policy that addresses GOF research involving the generation of pathogens with pandemic potential.

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[7] *United States Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses*. U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

[8] Ibid.

2. NSABB Charge

On October 22, 2014, as part of the USG's deliberative process for GOF studies, the NSABB was issued its charge to:

1. Advise on the design, development, and conduct of risk and benefit assessments for GOF studies, and
2. Provide recommendations to the U.S. government on a conceptual approach to the evaluation of proposed GOF studies

In developing its recommendations the NSABB was asked to consider: the results of the risk and benefit assessments; the discussions hosted by the National Academies; the spectrum of potential risks and benefits associated with GOF studies; and any alternative methods that may be employed to yield similar scientific insights or benefits, while reducing potential risks.

Since gain-of-function studies encompass a broad spectrum of pathogens and experimental manipulations, the NSABB discussed its charge and sought to identify the appropriate scope for its deliberations. Since the experiments that initiated the controversy involved the generation of pathogens that were concerning from a human health perspective, NSABB deliberations and recommendations focus on pathogens that pose risks to human populations. NSABB recommendations also focus on guiding USG funding decisions but the Board also considered issues associated with non-federally funded research and international research.

3. NSABB Deliberative Approach

The deliberative process (Figure 1) initiated by the USG to evaluate the risks and benefits of GOF studies involved the NSABB and the National Academies. To address its charge, NSABB formed two working groups to develop draft recommendations, which were then discussed by the full Board[9]. The National Academies convened public forums to stimulate broad discussions and receive additional stakeholder input. The first forum was held early in the deliberative process and a second was held in March 2016; both were designed to inform NSABB deliberations.

To inform the deliberative process further, NIH commissioned two additional analyses: 1) qualitative and quantitative risk and benefit assessments of GOF research, conducted by Gryphon Scientific, and 2) a review of the ethical considerations associated with the GOF issue and an analysis of relevant ethical decision-making frameworks, conducted by Dr. Michael Selgelid.

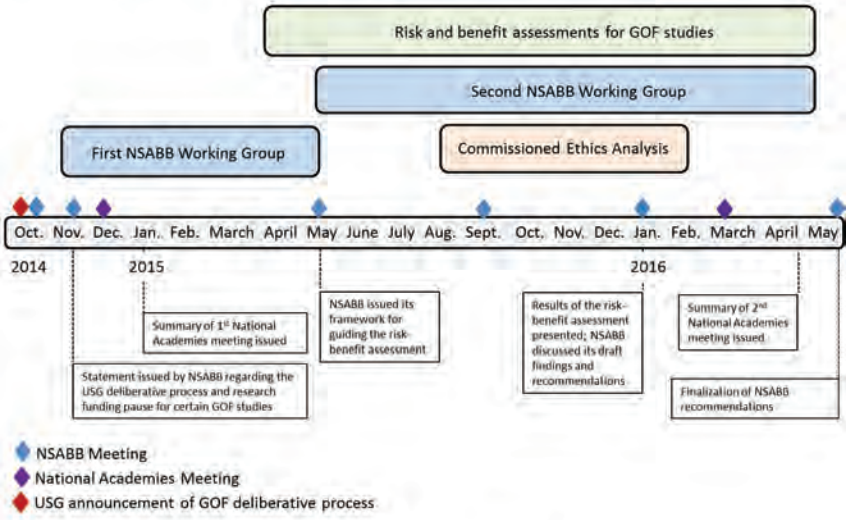

Figure 1. Timeline and major events of the GOF deliberative process.

[9] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/past-meetings

The NIH Office of Science Policy, which administers the NSABB, managed the overall deliberative process. NIH oversaw the work of its contractors, Gryphon Scientific and Dr. Michael Selgelid, and interfaced between the NSABB and contracted entities.

More information regarding the process and NSABB deliberations may be found in Appendices. Appendix A provides a detailed description of the NSABB's deliberative approach. Appendix B summarizes the current U.S. policy landscape for the oversight of pathogen research. Appendix C describes examples of studies that would or would not be considered GOF research of concern. Appendix D provides an overview of stakeholder views that were presented and considered by NSABB. Appendix E lists the experts and sources consulted by NSABB, including those who submitted public comments. Appendix F and G list the NSABB roster and charter. NSABB's *Framework for Guiding the Conduct of Risk and Benefit Assessments of Gain-of-Function Research*, which was approved by the Board in May 2015, is provided in Appendix H.

Guiding Principles for NSABB Deliberations

Early in the overall process the NSABB developed the principles below to guide its deliberations and underpin its analysis of the risk and benefit assessments.

1. The NSABB deliberations should focus on defining the GOF problem then include broad consideration of possible solutions. A range of approaches and decision-making frameworks will be considered, and the NSABB will take into account these various approaches when developing its recommendations.

2. NSABB will consider the potential risks and benefits of a broad range of GOF studies involving influenza, SARS, and MERS viruses in order to identify those that may raise significant concerns that should be addressed. However, the NSABB will aim to develop recommendations that are grounded in broadly-applicable concepts and principles that could, if necessary, apply to GOF studies involving other pathogens that may require evaluation in the future.

3. Similarly, NSABB will consider the risks and benefits associated with alternative research approaches to GOF research to understand whether or not these may substitute for or complement GOF studies.

4. NSABB recommendations will be informed by data and information about potential risks and benefits as well as values that will guide the evaluation and comparison of these risks and benefits. Ethical, societal, and legal considerations will also contribute to the development of recommendations and these inputs should be explicitly identified, discussed, and prioritized.

5. NSABB recognizes that not all analyses relevant to its task are quantitative and that uncertainties inherent in any quantitative analysis may remain. NSABB will seek to document important areas of uncertainty in any data or analysis when necessary.

6. NSABB should publicly debate its draft recommendations and describe in its report any dissenting views that may vary substantially from the Board's recommendations.

7. NSABB should consider current USG policies and guidelines, determine whether they adequately address risks associated with GOF research (in light of potential benefits), and make recommendations that are consistent with that determination. Current policies may be adequate or require only minor changes; alternatively, significant enhancements may be needed. The adequacy of current policy to cover GOF studies may vary by pathogen. Recognizing the paramount importance of ensuring safety, security, and public health, policies should also minimize the burdens placed upon the conduct of science.

8. NSABB recommendations will inform the development of U.S. government policy, which will apply to research funded, conducted, or overseen by the U.S. government either domestically or internationally. NSABB will be mindful in its deliberations of the likelihood that the Board's recommendations and U.S. policy decisions will also influence other governments and non-USG funders of life sciences research.

9. The NSABB will also consider whether there are certain studies that should not be conducted under any circumstances, and if so, articulate the critical characteristics of such studies.

10. Maintaining public trust and confidence in life sciences research is critical and must be taken into account as recommendations are formulated.

4. Analysis

In developing recommendations on a conceptual approach for evaluating GOF proposals, NSABB examined three major areas: the current policy landscape for overseeing research involving pathogens, the ethical issues associated with funding and conducting GOF studies, and the results of the risk and benefit assessments of GOF research. In addition, the NSABB considered broad stakeholder perspectives through presentations from domestic and international experts at working group and full NSABB meetings, analysis of published articles, and comments from attendees at NSABB meetings or public comments submitted to the Board.

4.1. Analysis and Interpretation of the Risk and Benefit Assessment

The NSABB reviewed the risk and benefit assessments conducted by Gryphon Scientific, which were designed to evaluate the risks and benefits of GOF research in a manner that encompassed both benign and worrisome aspects of a broader range of GOF studies than those that have raised concerns. The RBA analyzed biosafety and biosecurity risks as well as possible benefits. Overall, the RBA includes a commendable amount of sophisticated work and analysis, is generally well-done, and largely achieves the goals it was intended to address. Gryphon's draft RBA report was made publically available in December 2015 and key results were presented and discussed at NSABB and National Academies meetings. The final report was issued in April 2016 and is available on Gryphon's website.[10]

Strengths of the Risk and Benefit Assessments

The RBA has significant strengths. It is a thorough and extensive analysis of the risks and benefits of GOF work in the context of the guidance provided in the NSABB *Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research*.[11] It takes into account the principles articulated in the framework and includes the agents, categories of possible risks, types of possible benefits, and possibly concerning scenarios and phenotypes that were laid out in the *Framework*. It was agreed that a few items from the *Framework* not be analyzed or focused on in the RBA so that the most probable issues of concern could be thoroughly addressed within the available time and resources.[12]

The biosafety risk assessment does a credible job of defining the relative risks associated with potential laboratory accidents involving GOF manipulations of pathogens with enhanced characteristics as compared to wild-type pathogens. This analysis is performed in a semi-quantitative way; it uses

[10] *Risk and Benefit Analysis of Gain-of-Function Research, Final Report*. Gryphon Scientific, April 2016. http://www.gryphonscientific.com/wp-content/uploads/2016/04/Risk-and-Benefit-Analysis-of-Gain-of-Function-Research-Final-Report.pdf

[11] *Framework for Conducting Risk and Benefits Assessments of Gain-of-Function Research.* National Science Advisory Board for Biosecurity, May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

[12] National Science Advisory Board for Biosecurity Meeting, May 5, 2015. http://osp.od.nih.gov/office-biotechnology-activities/event/2015-05-05-120000-2015-05-05-200000/national-science-advisory-board-biosecurity-nsabb-meeting

appropriate, established, peer-reviewed methods to the extent available. The parametric approach employed is powerful and allows consideration of many situations of interest.

The report effectively illustrates that the harmful events being modeled are low probability (see Figures 6.2 and 6.4 in Gryphon's report). Only a small fraction of laboratory accidents would result in a loss of containment. Of those, only a small fraction would result in a laboratory acquired infection, and of those, only a fraction would spread throughout the surrounding community (or to the global population). The NSABB recognizes the challenge of analyzing low-probability, high-consequence events for which little data exists and appreciated attempts to make this point clear in the RBA.

The biosecurity risk assessment is primarily qualitative, and highlights analysis of previous malevolent events and evasions of security systems, likely capabilities and motivations of various possible actors, and an evaluation of the systems in place to prevent biosecurity breaches. Information was obtained from a survey of literature and discussions with biosecurity, intelligence, and law enforcement professionals. It is an extensive gathering of a wide range of information that has not been presented before in one place.

The information risk assessment (an element of the biosecurity risk assessment) is a qualitative analysis of risks that may result from the misuse of information derived from certain GOF studies that might be published in the future. It identifies information that might be attractive to malicious actors and compares it to other sources of information they might find attractive.

The benefits assessment uses a novel approach to assess potential benefits of GOF studies, a difficult task with little prior methodology to draw upon. The results are not quantitative, and attempts at quantification would have been appreciated. However, as is, the assessment may be the best that can be done with the available information and analytic tools. The benefits assessment thoroughly analyzes the possible benefits of alternatives to GOF studies and identifies areas where GOF research appears to provide unique benefits (i.e., benefits that are not attainable without the use of GOF), either currently or in the near future.

The RBA contains a number of other useful analyses as well, including background and contextual information on the biology of influenza and coronavirus, historical analysis of naturally-occurring seasonal and pandemic influenza and coronavirus outbreaks, an examination of the potential proliferation of GOF research, and analysis of the potential loss of public trust in science that could result if a laboratory incident involving GOF research were to occur. Significantly, the historical analysis notes that each year, influenza infects 5 – 10% of the world's population, resulting in significant morbidity and mortality (up to 500,000 deaths per year). This description of naturally-occurring influenza (and coronavirus) infections helps to establish the extant risks associated with these infectious diseases to which the risks associated with GOF studies might be compared.

Overall, the RBA is comprehensive, objective, reasonable, and generally extensively documented.

Limitations of the Risk and Benefit Assessments

The RBA also has some weaknesses and limitations that should be noted. First, the RBA was limited to the types of labs traditionally funded by the Federal government, which may not be representative of other settings where GOF research may be conducted. Every attempt was made to base the analyses in the RBA on scientific information and data. Nevertheless, data on the properties of the various pathogens being examined, events such as laboratory accidents or security breaches, or possible future acts of terrorism, are limited in some cases and unavailable in principle in others. Therefore, assumptions and estimations were necessary. For this reason, the biosafety risk assessment is not fully quantitative, primarily because absolute, quantitative baselines for the risk of work with wild-type pathogens could not be estimated with any certainty. Thus, the data presented are primarily comparative, and provide relative, rather than absolute, values for the risks associated with laboratory accidents involving GOF studies.

Gryphon compared the risks associated with potential lab accidents involving a GOF strain with the risks associated with the same accident involving a wild-type strain. This comparative approach is adequate for some scenarios but inadequate for others. For example, an increased risk associated with a GOF study that is relatively large (5-10-fold or greater) may appear significant, but if this increase is in comparison to a very small risk baseline, the overall risk associated with the GOF study may not be significant or concerning. Similarly, small increases in risk over a higher risk baseline, in fact, may be concerning. Additionally, differences in risk that are relatively small (about 2-fold) are difficult to interpret because such changes may fall within the limits of uncertainty for the analysis. Attempts to include some absolute baseline estimates of risk (an admittedly difficult task) were included in Section 6.8 of Gryphon's report. However, the lack of comprehensive estimates of baseline risk make interpreting the biosafety risks a challenge.

Given the comparative approach undertaken for the biosafety risk assessment, the implications of the results of this analysis depend a great deal on the wild-type comparator strains that were selected for the analysis. For instance, for pandemic influenza Gryphon initially selected the 1918 influenza strain as the comparator. Gryphon regarded this strain as embodying the maximum risk for influenza, yet a level of risk that is also deemed as acceptable given that research with this strain is permitted. However, using 1918 influenza as the comparator for the analysis compares GOF risks to a relatively high level of baseline risk, making the changes in risk associated with GOF manipulations comparatively small. Utilizing different comparator strains alters the relative risks associated with GOF manipulations. Using a high-risk baseline strain may obscure significant risks associated with GOF studies whereas using a strain with a low risk baseline may inflate the potential risks associated with GOF studies.

Little data exists about the probabilities of the accidents that initiate the chain of events that may lead to a pandemic and therefore, the quantitative probability of these accidents could not be incorporated into the biosafety risk assessment. The modeling of secondary spread of a pathogen through populations once it is released from a laboratory allows for some estimation of the consequences of an event, but without a better understanding of the likelihood that an accident would result in loss of containment or a laboratory acquired infection it is difficult to make judgments about the overall risk.

Gryphon's analysis accounts for this by presenting relative, actuarial risk. However, this approach results in the challenges associated with comparing relative risks described above. There are large uncertainties in most of the input parameters that are the basis for the biosafety risk calculations. Uncertainties about inferring absolute risk from these relative risks exist and were kept in mind as the Board developed its findings and recommendations.

The biosecurity risk assessment attempts to examine how GOF studies add to the risk of malevolent acts. Portions of the biosecurity risk assessment focus on GOF studies but others describe the type of threats that could occur against any high-containment laboratory. The semi-quantitative portion of the biosecurity risk assessment estimates probabilities for escape and secondary spread and escape from local control for various pathogens and event types. However, this analysis (see section 7.4 and Table 7.7 in Gryphon's report) assumes that 1, 5, or 10 individuals are initially infected as a result of a malicious act with no indication of how likely such an event would be, since there is no way to make such an estimate.

While exhaustively documented, the RBA is not always transparent about data reliability. In particular, interviews were used to gather much critical information, and this was not always well documented in a way that reflects how robust the resulting information may be. For peer-reviewed publications, this is less of a concern.

While evaluation of the benefits of alternatives to GOF studies is extensive, evaluation of risks of alternative approaches is not as thorough. In addition, risks and benefits are not presented in comparable terms, making it a challenge to determine whether certain risks are justified by potential benefits. Significantly, the benefit assessment is not quantitative and there is no probability analysis or attempt to estimate the likelihood that a certain benefit would be realized or what its impact might be.

Key Results of the Risk and Benefit Assessments

While NSABB considered all of the analyses in the RBA, some results are important to highlight. In general, the RBA examined risks and benefits associated with the major GOF phenotypes with the intention of identifying types of studies that would be most and least concerning, based particularly on their risk profile.

With regard to biosafety risks, only some potential GOF phenotypes represent substantially increased (5- to 10-fold or more) risks over the starting strain. Two-fold changes most likely fall within the uncertainty of the data, and while small differences might be important if it could be shown that they are significant, this demonstration is probably difficult. For coronaviruses, GOF studies that would create strains with increased transmissibility among mammals may entail significant risks if they also increase human transmission. The risks, were this combination to occur, would include increased probability of an outbreak escaping local control and increased likelihood of global consequences. In addition, experiments that enhance coronavirus growth in culture would likely increase the possibility of laboratory acquired infections.

For seasonal influenza, the GOF phenotypes entailing the greatest risks include enhanced transmission in mammals (assuming this increases transmission in humans), enhanced virulence, and evasion of immunity. Enhanced pathogenicity might significantly increase the global consequences of an outbreak.

For pandemic influenza, the issue of what GOF phenotypes could increase risk is highly dependent on the comparator strain used. If 1918 influenza is modified so that it is able to evade residual immunity, it could become more of a threat than 1957 H2N2, the comparator Gryphon used. For 1957 H2N2, enhancement of pathogenicity to that of 1918 also significantly increases risk. Other phenotype changes had little effect. However, if less transmissible and/or less virulent pandemic strains were used as the basis for comparison, the risks of some other GOF studies would appear to increase risk more significantly.

For avian influenza, the GOF experiments that lead to enhanced transmissibility in mammals (and presumably humans) would likely lead to an increased probability of local and widespread outbreaks, as well as increased global consequences. More subtle aspects of these very general conclusions may be found in the biosafety risk section and the Executive Summary of Gryphon's RBA report.

In general, GOF studies that were not considered by the NSABB to entail significant risks were those that would: adapt human pathogens to mammals to generate animal models; enhance the growth of attenuated vaccine strains; and antigenic drift studies that are commonly used to guide vaccine selection.

The biosecurity risk assessment shows that the most probable threats involve insiders who have direct access to dangerous pathogens or outsiders who collaborate with or subvert insiders. If currently mandated biosecurity systems are effective, outsiders have little chance of causing harm on their own. The RBA report also concludes that the risks associated with information from future GOF studies with influenza, SARS or MERS appear small; this is because most of the information of interest is already published, or non-GOF information relating to pathogens that are more attractive to individuals with malevolent intent is readily available. However, future scientific advancements could alter this assessment.

Most GOF studies provide benefits in the form of new scientific knowledge, and some of these benefits are unique (i.e., unable to be achieved by alternative, non-GOF approaches). While some GOF studies are likely to provide unique near-term benefits, these are associated with specific agents and phenotypes. With regard to more applied benefits, such as countermeasure development and biosurveillance, the most clear-cut example is experiments that increase growth of seasonal influenza vaccine candidates in culture. These studies provide unique benefits to current production of seasonal influenza vaccines, and likely will in the future. Another reasonably clear unique benefit is derived from experiments that enhance mammalian pathogenicity of coronaviruses as a means of developing animal models for studying disease and developing countermeasures. GOF studies that yield phenotypes that provide unique benefits to countermeasure development include enhanced pathogenicity, evasion of vaccines, and evasion of therapeutics. For several other potential benefits of GOF studies involving seasonal influenza, either the potential benefit is long term, or alternative approaches may yield the

same or similar benefits. Interestingly, few unique benefits pertaining to GOF studies involving pandemic influenza were identified. There are several types of GOF studies that entail generating avian influenza strains with phenotypes that may be valuable for surveillance and preparedness efforts, although other advances are needed to fully realize such benefits. This point is controversial, with strong proponents and critics. Additionally, a variety of benefits of GOF studies were identified that may also be provided to some extent by alternative approaches. It should be noted that no attempt was made to provide a probability assessment based on historical data for potential benefits; hence no direct comparison of risk to benefit for a proposed research project is possible.

4.2. Consideration of Ethical Values

The RBA provides information about the potential risks and benefits associated with conducting GOF research, however, determinations about whether such studies should be undertaken involve value judgments based on weighing the risks and benefits. The NSABB identified a number of values that are applicable to the decisions about whether to fund certain GOF studies and how to oversee them. Sources of these values include the Belmont Report,[13] the literature on public health ethics,[14] and the literature on oversight of emerging technologies,[15] as well as the literature specifically debating appropriate approaches to overseeing dual use research of concern (DURC) and GOF research that has raised concerns.[16,17,18,19,20] The commissioned ethics analysis conducted by Dr. Michael Selgelid describes additional values as well as decision-making frameworks to be considered.[21]

Substantive values

The following values are important to consider when determining whether to fund a research proposal involving GOF studies that might entail significant risks.

Non-maleficence: not causing harm. There are inherent risks associated with research involving pathogens that could result in harm to individuals as a result of accidental or intentional infection

[13] The Belmont Report. Office of the Secretary, U.S. Department of Health and Human Services. *Ethical Principles and Guidelines for the Protection of Human Subjects Research*. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979. http://www.hhs.gov/ohrp/humansubjects/guidance/belmont.html

[14] Kass, N.E., *An Ethics Framework for Public Health*. American Journal of Public Health. 2001; 91(11):1776-1782. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1446875/

[15] *New Directions. The Ethics of Synthetic Biology and Emerging Technologies*. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf

[16] Resnik, D.B., *H5N1 Avian flu research and the ethics of knowledge*. Hastings Center Report 2013; 43, 2: 22-33.

[17] Kelle, A., *Beyond patchwork precaution in the dual-use governance of synthetic biology*. Sci Eng Ethics. 2013 Sep; 19(3):1121-39.

[18] Kuhlau, F., Höglund, A.T., Evers, K., Eriksson, S., *A precautionary principle for dual use research in the life sciences*. Bioethics. 2011 Jan; 25(1):1-8.

[19] *Biotechnology Research in the Age of Terrorism*. The National Academies, 2004. http://www.nap.edu/catalog/10827/biotechnology-research-in-an-age-of-terrorism

[20] *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information*. National Science Advisory Board for Biosecurity, June 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf

[21] Selgelid, M., *Gain-of-Function Research: Ethical Analysis*. April 2016. http://osp.od.nih.gov/sites/default/files/Gain_of_Function_Research_Ethical_Analysis.pdf

with the pathogen. These might include: causing disease; loss of lives; damage to the economy, national or international security, or agriculture; or loss of public trust in science or governance structures. Approaches aimed at preventing harm and mitigating potential risks should be considered and applied to the design, conduct, and communication of GOF research involving pathogens.

Beneficence: promoting beneficial outcomes while preventing harmful outcomes; appropriately balancing benefits and risks; formulating policy that maximizes public benefit while minimizing public harm. Benefits might include: preventing disease; saving lives; improving public health; enhancing the economy, national and international security, or public trust in science and governance structures. When the ultimate goals of the research are to improve public health, public health ethics would consider how effective the research is likely to be in achieving those goals, what the known or potential burdens of the research are, whether those burdens can be minimized, whether there are alternative approaches that are less risky or burdensome, and how the potential benefits and burdens of the research can be fairly balanced. The work of the Presidential Commission for the Study of Bioethical Issues (Bioethics Commission) suggests that those formulating and implementing government policy on scientific research and emerging technologies have a duty of public beneficence – a duty "to promote individual activities and institutional practices...that have great potential to improve the public's well-being," while being "vigilant about risks and harms, [and] standing ready to revise policies that pursue potential benefits with insufficient caution."[22] Both risks and benefits have associated probabilities, magnitudes, and uncertainties. In some instances, it may be justifiable to pursue benefits despite the potential risks; in others, the potential benefits may be foregone due to possible risks.

Social justice: distributing potential benefits and harms fairly (distributive justice) and selecting participants in research fairly, as well as those who may potentially be exposed to risk. There are many different approaches to social justice, such as egalitarianism, utilitarianism, and libertarianism,[23] to name a few. Decisions about whether to fund research that entails some risk should consider how the risks and benefits associated with conducting that research will be distributed, with an effort to distribute risks and benefits as fairly as possible. When considering pandemic potential, fair distribution of risks and benefits must be considered on a global scale.

Respect for persons: allowing competent individuals to make informed choices, and ensuring that the representatives of individuals lacking capacity to choose can make choices in keeping with the wishes, values, or interests of those represented. Autonomy generally requires informing human research participants, laboratory workers, and the public about the risks of research and eliciting their free and uncoerced decision about whether to subject themselves to those risks. In the case of the public, mechanisms for representative decision-making and publicly accountable governance may be needed, as getting consent directly from the members of the public may be impracticable.

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[22] *New Directions. The Ethics of Synthetic Biology and Emerging Technologies*. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf

[23] Nozick, R., *Anarchy, State, and Utopia*. New York: Basic Books, 1974.

Scientific freedom: avoiding unnecessary interference with scientific research, debate, or publication. Scientific freedom includes an entitlement to avoid interference unless necessary (negative freedom), but not the affirmative right to receive funding or other forms of support for a particular project (positive freedom). Scientific freedom implies a duty of compliance with norms and regulation to promote the responsible conduct of research and protect participants in research and the public. As a corollary to the principle of scientific or intellectual freedom, the Bioethics Commission endorses a principle of regulatory parsimony, requiring "only as much oversight as is truly necessary to ensure justice, fairness, security, and safety while pursuing the public good."[24]

Responsible stewardship: acting in a way that shows concern for children, future generations, and the environment. The Bioethics Commission emphasizes that this is both a domestic and global responsibility that requires "prudent vigilance, establishing processes for assessing likely benefits along with assessing safety and security risks both before and after projects are undertaken."[25]

Procedural Values

The following values apply to the process of decision-making about GOF research and are important to consider when establishing mechanisms to review and/or approve the funding of research proposals involving GOF studies that may entail significant risks.

Public participation & democratic deliberation: making decisions with participation from the public, utilizing respectful debate and inclusive deliberation. Life sciences research is largely a publicly-supported endeavor; therefore, those who allocate funds and conduct life sciences research have a responsibility to be good stewards of public funds and to respond to the interests and concerns of the public. Many, if not all, members of society have a stake in the research enterprise and will be affected directly or indirectly by the benefits and risks stemming from such research. This stakeholder community has diverse values and tolerances for risk, which are important to consider when making decisions about funding and overseeing life sciences research. Some forms of public participation include: oversight by the legislative or executive branches of government, public membership and input on government science advisory committees, other mechanisms of public governance, surveys of public opinion on science policy issues, research models such as community-based participatory research, and efforts by scientists and government officials to share information with the public and better understand the public's interests and concerns. The Bioethics Commission urges the importance of democratic deliberation, as "[a]n inclusive process of deliberation, informed by relevant facts and sensitive to ethical concerns, promotes an atmosphere for debate and decision making that looks for common ground wherever possible and seeks to cultivate mutual respect where irreconcilable differences remain."[26]

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[24] *New Directions. The Ethics of Synthetic Biology and Emerging Technologies*. Presidential Commission for the Study of Bioethical Issues, December 2010. http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf, p5.
[25] Ibid., p5.
[26] Ibid., p5.

Accountability: taking responsibility for one's actions and being prepared to justify or explain them to others. It is important that decisions to fund research are justifiable to the public and others. Decisions should be justified in terms of substantive and procedural values.

Transparency: sharing with the public the information and assumptions used to make decisions, including uncertainties, controversies, and limitations of analyses. Transparency is an important part of accountability and public participation. It also allows review and reconsideration of policy over time as new facts emerge and analysis evolves.

4.3. Decision-Making Strategies and Frameworks for Evaluating and Managing Risks and Developing Policy

The NSABB identified a number of approaches or frameworks that may be used to guide the making of complex decisions with ethical implications, particularly in the face of uncertainty. These may also be used in developing policies for managing GOF research. Different strategies reflect different attitudes toward risk-taking and some may be more appropriate in some situations than others. The NSABB examined a number of such strategies as it attempted to determine the best option(s) with respect to GOF research that has raised concerns. These options are not mutually exclusive, and elements from more than one may be used together to develop a path forward. The following are decision-making frameworks that were considered.

Maximax: choosing the option with the best possible outcome. Maximax is a relatively simple strategy that focuses on choosing the option with the best possible outcomes. While maximax may be appropriate for making some types of personal choices (e.g. playing games with nothing of value to lose), it may not be appropriate for making science and technology policy decisions because most people would want to take appropriate steps to prevent or mitigate risks regardless of benefits. For GOF studies, use of maximax would involve identifying research with the best possible benefits, regardless of risks.

Maximin: choosing the option with best outcome among the worst possible outcomes. Maximin is a risk-averse approach because it aims to avoid the worst possible outcomes. Maximin is another relatively simple approach, but may present difficulties when applied to making science and technology policy decisions, because it would recommend not developing a new technology if this decision could lead to the worst possible outcome. Since all technologies (and scientific ideas) can conceivably lead to good and bad outcomes, strict adherence to maximin would result in a very cautious approach to science and technology development. For GOF studies, use of maximin would involve identifying studies with risks, and choosing the least risky regardless of benefits.

Expected Utility Theory: choosing the option that maximizes expected utility, where expected utility for a possible outcome = probability x utility. Expected utility theory involves a quantitative balancing of risks and benefits and is inherently a more complex process. Cost-benefit analysis in economics is a form of expected utility theory. A problem with expected utility theory is that sufficient evidence may not always be available to confidently estimate the probabilities involved in

the utility calculus. When this is the case, other approaches may be appropriate. For GOF studies, use of expected utility theory would require quantitatively determining the likelihood of risks and benefits and calculating the resulting utility.

Precautionary approach: involves taking reasonable measures to prevent, minimize, or mitigate risks that are significant and plausible. A measure is "reasonable" if it: 1) appropriately balances the values at stake in the risk management; 2) is proportional to nature of the risk (i.e. greater risks require stronger measures); and 3) is likely to be effective. A risk is "plausible" if there is some scientific evidence that it could occur even if the probability of the risk cannot be confidently estimated. There are many versions of the precautionary principle, including ones that are more or less risk-averse.[27, 28] A precautionary approach, in general, would limit an activity unless the environment, health, or security, are clearly protected. This approach can recognize a potential problem early and prevent harm from occurring but may lead to regulatory burdens or unnecessarily limit activities. This approach might restrict potential GOF research unless the studies are demonstrated to be safe.

Permissive approach: in general, this would allow an activity unless the environment, health, or security, are clearly compromised. This approach may reduce unnecessary regulatory burdens but can result in after-the-fact reaction to harms. This approach might allow certain GOF studies to proceed until they are demonstrated to entail significant risk.

Planned adaptation or risk-based approach: provides a systematic way to deal with managing risks in the face of uncertainty. It involves: 1) preparation to identify the risks and regulatory gaps, including input from a broad range of perspectives; 2) putting measures in place to control risk based on the best information available at the time; 3) systematically gathering data and observing the effects of policies; and 4) updating and revising policies as needed. An example of an adaptive approach is the life cycle approach taken by the Food and Drug Administration when making decisions about whether to approve drugs, when that includes post-market surveillance.[29] For GOF studies, this approach might entail allowing studies that raise concerns to proceed under defined conditions, then evaluating the risk-benefit landscape periodically to determine whether the studies that are permitted should continue, be expanded, or be restricted.

Threshold approach: identifying a risk threshold beyond which, certain studies are given special attention or subject to additional scrutiny or oversight and might preclude certain studies. Implementation would involve defining or describing the studies that would require additional oversight as well as a description of what that oversight would entail. This approach would allow for the identification of studies of concern but might need to be reevaluated if the risk landscape changes and the threshold that was identified is no longer appropriate. For GOF studies, this would

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[27] Resnik, D.B., *Environmental Health Ethics*. New York: Oxford University Press, 2013.
[28] Munthe, C., *The Price of Precaution and the Ethics of Risks*. Dordrecht: Springer, 2011.
[29] FDA determinations about whether a new drug is safe and effective are complex, address uncertainty, and involve ongoing monitoring to assess risks and benefits and take appropriate post-marketing actions as necessary. See: *Structured Approach to Benefit-Risk Assessment in Drug Regulatory Decision-Making*, 2013.
http://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM329758.pdf

entail identifying the characteristics of studies involving significant risks that may not be adequately managed and then stipulating further oversight or determining that they should not be conducted.

Point-source approach: involves controlling where certain studies are conducted and under what conditions. This approach would centralize certain research activities, restricting them to designated locations or facilities. For GOF studies that raise concerns this might involve requiring that certain studies only be conducted in facilities with certain biocontainment conditions, biosafety practices, and security measures.

The NSABB used ideas from a number of frameworks to inform its findings and deliberations (Sections 5 and 6). The criteria for identifying GOF research of concern (GOFROC) (see Recommendation 1) reflect a threshold approach. The principles for guiding funding decisions for GOFROC include elements from several of the decision frameworks described above. For instance, an explicit call for a risk-benefit analysis (Recommendation 1, Guiding Principle 3) reflects expected utility theory; however, a strictly quantitative calculation is probably not possible. The principles to guide funding decisions that call for risk mitigation and a restriction to laboratories with a demonstrated capacity to safely carry out the studies (Recommendation 1, Guiding Principles 4, 5 and 7) incorporate elements of point-source and precautionary approaches. An adaptive approach was considered particularly attractive and appropriate for policies aimed at providing oversight of GOF research (Recommendation 3).

4.4. Examination of the Current Policy Landscape

Many U.S. government agencies fund life sciences research in furtherance of their specific missions. In general, research supported by the USG is founded on the principle of scientific merit and goals of the funding agency. Multiple complementary layers of oversight are in place to manage laboratory and other risks associated with federally-funded life sciences research. These policies are intended to provide oversight at various points throughout the research life cycle, from research conception to its publication and translation into practice. These policies include a foundation of occupational health and medicine (for laboratory and clinical workers), laboratory biosafety practices, and policies that address biosecurity risks. Below is a description of the oversight policies in place for federally-funded life sciences research involving pathogens, with discussion of whether and how such policies apply to GOF studies. This analysis is illustrated in Figures 2 and 3 and summarized in Appendix B.

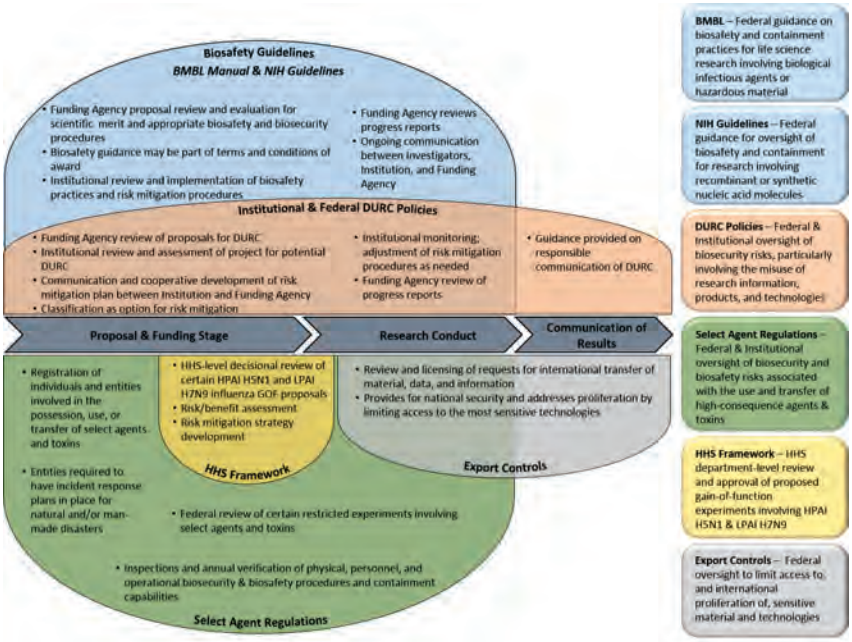

Figure 2. U.S. government oversight of life sciences research involving pathogens. Oversight policies apply at different stages and occur at different levels throughout the research life cycle. See text and Appendix B for descriptions of each policy. These policies have different applicability and scopes and therefore do not apply to all life sciences (or GOF) research projects.

Scientific Merit Review

Departments and agencies within the U.S. government fund diverse portfolios of life sciences research. Funding decisions are based on the scientific merit of a given proposal and the ability of a project to advance the agency's strategic mission. The USG funds life sciences research through a variety of mechanisms including grants, contracts, and cooperative agreements. Each funding agency has its own processes for evaluating research proposals and awarding funds but, in general, proposals are subject to rigorous scientific review by Federal agency staff and often, scientific peers. NIH grant proposals, for example, undergo two levels of review. The first evaluation is by a panel of scientific peer reviewers who score proposals based on scientific merit and other criteria. The second round of review includes discussion of meritorious proposals at public meetings of advisory councils, specific to individual funding institutes and centers within NIH, to determine how proposals fit within their broader strategic objectives.

Biosafety Oversight

Oversight of pathogen research focuses first on ensuring the safe handling of biological agents through appropriate biosafety practices and containment measures, which are addressed by the *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*)[30], the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)[31], and other documents. The BMBL and the *NIH Guidelines* provide for federal and institutional biosafety oversight and guidance involving biosafety practices and containment features that are based on risk assessments for specific projects. Such determinations are typically made at the institutional level and are guided by federal guidelines and policies, which are updated as necessary to provide additional guidance for research involving emerging pathogens or technologies. Biosafety is achieved by conducting research under appropriate physical and biological containment levels and employing practices that help to ensure a safe working laboratory environment.

The BMBL is a guidance document developed by CDC and NIH that is generally considered the authoritative reference for laboratory biosafety in the United States. The BMBL provides summary statements for many bacterial, fungal, parasitic, rickettsial, viral, and other agents. These statements describe the characteristics of the pathogen, its natural mode of infection, potential occupational hazards with the agent, and recommendations for laboratory safety and containment. It also describes the fundamentals of biological containment, which include descriptions of proper microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. It describes the process of biological risk assessment, which enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections. It also describes occupational health, immunoprophylaxis, and principles for laboratory biosecurity. The BMBL is updated periodically to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and public health.

> **Analysis:** The BMBL does not address GOF studies *per se* but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update, but interim laboratory biosafety guidance was issued by CDC.[32]

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[30] *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th Edition. http://www.cdc.gov/biosafety/publications/bmbl5/

[31] *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines), November 2013. http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

[32] *Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2.* http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html [last updated June 18, 2015]

The BMBL is not a regulatory document. U.S. funding agencies may require it be followed as a term and condition of funding but, in general, compliance with the BMBL is voluntary. In addition, the BMBL provides general biosafety guidance but does not describe detailed procedures or experiment-specific containment protocols.

The *NIH Guidelines* specify the practices for safely constructing and handling: recombinant nucleic acid molecules; synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules; and cells, organisms, and viruses containing such molecules. The *NIH Guidelines* apply to basic and clinical research involving recombinant or synthetic nucleic acid molecules conducted at or sponsored by institutions that receive NIH funding for any such research. Compliance with the *NIH Guidelines* is required by NIH as a term and condition of award of funding. Other USG agencies may also require compliance with the *NIH Guidelines*.

The *NIH Guidelines* focus on the concepts of risk assessment, risk group classification of agents based on their ability to cause disease in humans and the availability of medical countermeasures, physical and biological containment levels, practices, personal protective equipment, and occupational health. To help ensure the safe conduct of this research, the *NIH Guidelines* specify roles and responsibilities of investigators and institutions. Institutions subject to the *NIH Guidelines* must establish Institutional Biosafety Committees (IBCs) composed of members with appropriate expertise, to review and approve such research. IBCs provide local oversight and ensure compliance with the *NIH Guidelines*. Certain higher risk experiments require review by the Recombinant DNA Advisory Committee (RAC)[33] and specific approval by the NIH Director as Major Actions. These experiments involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine or agriculture.

In order to continue to provide appropriate guidance for emerging pathogens or experimental approaches, the *NIH Guidelines* are updated periodically. The *NIH Guidelines* have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1), to specify enhancements to biosafety level 3 containment, practices, and to incorporate occupational health requirements. In 2012, the *NIH Guidelines* were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains that are transmissible among mammals by respiratory droplets.

Analysis: The *NIH Guidelines* provide guidance on risk assessment and appropriate containment and practices for conducting research involving recombinant or synthetic nucleic acids, which would

[33] The Recombinant DNA Advisory Committee (RAC) is a federal advisory committee that provides recommendations to the NIH Director related to basic and clinical research involving recombinant or synthetic nucleic acid molecules. See: http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/rac

apply to most government-funded GOF research. Some IBCs also review and approve non-recombinant pathogen research, however, not all institutions require their IBCs to do so. While the *NIH Guidelines* are often used as a model of biosafety guidance by the broader scientific community, compliance is required only by institutions receiving funding from the NIH for research involving recombinant or synthetic nucleic acid molecules. Therefore, some GOF studies may not be subject to the *NIH Guidelines* depending on whether the institution where the research is being conducted is subject to the *NIH Guidelines*.

The Federal Select Agent Program

The *Public Health Security and Bioterrorism Preparedness and Response Act of 2002*[34] requires the U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) to establish and regulate a list of select agents — biological agents and toxins that have the potential to pose a severe threat to public health and safety or animal or plant health or animal or plant products. The Federal Select Agent Program (FSAP) is administered jointly by the HHS Centers for Disease Control and Prevention and USDA Animal and Plant Inspection Service. The FSAP oversees the possession, use and transfer of biological select agents and toxins. The Select Agents and Toxins List is reviewed and updated biennially. Under the select agents regulations, individuals and institutions that possess, use, or transfer any select agent are required to be registered, follow appropriate biosafety procedures, and undergo periodic inspections. Individuals must be registered with the FSAP to have access to select agents or toxins, which requires that they undergo a security risk assessment performed by the Federal Bureau of Investigation (FBI). There are legal penalties for failing to comply with the select agent regulations.

In addition to the agents and toxins on the list, the select agent regulations apply to some genetic elements, including nucleic acids that are immediate precursors to infectious forms of any select agent viruses (i.e., complete positive strand RNA viral genomes), as well as some nucleic acids that encode select toxins. Select agent regulations also apply to genetically-modified select agents and toxins. Restricted experiments are described in the regulations and involve the deliberate transfer of or selection for a drug resistance trait to select agents that are not known to acquire the trait naturally. If the acquisition of resistance is to a first-line drug that could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, the restricted experiment requires special review and approval by the SAP. Some attenuated strains of select agents may be excluded from the regulations based upon a determination that the attenuated strain or modified toxin does not pose a severe threat to public, plant, or animal health or safety. The list of select agents and toxins is reviewed and updated biannually. The Intragovernmental Select Agent and Toxin Technical Advisory Committee serves as an advisory group to the FSAP and provides recommendations on the addition or deletion of agents or toxins to/from the select agent list. Following the disclosure of laboratory incidents at Federal

[34] *Public Health Security and Bioterrorism Preparedness and Response Act of 2002.* https://www.gpo.gov/fdsys/pkg/STATUTE-116/pdf/STATUTE-116-Pg594.pdf

facilities involving select agents in 2014, two advisory committees issued recommendations on ways to strengthen the FSAP.[35, 36] Plans to implement these recommendations are also in place.[37]

Analysis: GOF studies are subject to oversight by the FSAP if they involve pathogens on the select agent list. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the FSAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted. However, MERS-CoV is not a select agent. GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the FSAP (though they could be subject to other federal and institutional biosafety oversight). The FSAP is underpinned by a regulatory requirement that applies to non-USG funded (i.e., private sector funded) pathogen research as well.

Federal and Institutional Oversight of Life Science Dual Use Research of Concern

The U.S. government has issued policies for the oversight of life sciences DURC. These policies focus oversight on research involving 15 high-consequence pathogens and toxins[38] that involve seven categories of experimental activity, which are projects that can be reasonably anticipated to:

1. Enhance the harmful consequences of the agent or toxin;
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;
3. Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin;
5. Alter the host range or tropism of the agent or toxin;
6. Enhance the susceptibility of a host population to the agent or toxin; or
7. Generate or reconstitute an eradicated or extinct agent or toxin listed above.

[35] *Report of the Federal Experts Security Advisory Panel*. U.S. Government, December 2014.
[36] *Fast Track Action Committee Report: Recommendations on the Select Agent Regulations Based on Broad Stakeholder Engagement*, U.S. Government, October 2015.
[37] Lisa Monaco and John Holdren White House Memorandum, October 29, 2015, Next Steps to Enhance Biosafety and Biosecurity in the United States. https://www.whitehouse.gov/sites/default/files/docs/10-2015_biosafety_and_biosecurity_memo.pdf
[38] Section III of the *United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern* and Section 6.2.1 of the *United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern*. The agents within the scope of the USG DURC policies are the 13 Tier 1 select agents plus HPAI H5N1 and 1918 influenza virus.

Projects involving any of the 15 agents and that could be anticipated to involve any of these seven experimental effects are then determined to be DURC if they then meet the definition of DURC listed in the policy.[39]

The DURC policies outline a coordinated approach to oversight involving the federal funding agencies and institutions that conduct such research. The policy for federal oversight, issued in March 2012, requires Federal Departments and Agencies to review proposed and ongoing research projects to identify any that constitute DURC.[40] The policy for institutional oversight, issued in September 2014, articulates responsibilities of research institutions in identifying and managing DURC. Research institutions are to establish an Institutional Review Entity (IRE) to review research subject to the policy to determine whether any such research involves any of the seven experimental effects, and if so, whether the research constitutes DURC. IREs may review projects not specifically covered under the DURC policies but such additional reviews are voluntary.

When DURC is identified—either by a funding agency or a research institution—the funder and institution are to work collaboratively to develop a risk mitigation plan to help ensure that the research is conducted and communicated in a responsible manner. DURC risk mitigation plans are approved by the federal funding agency and are reviewed on an annual basis by the funder and the institution. Specific risk mitigation measures may be incorporated into a term of award. Risk mitigation may involve modifying the design or conduct of the research in order to address the same scientific question in a manner that poses fewer biosafety or biosecurity risks. Other measures may involve applying enhanced biosafety or biosecurity measures, evaluating the effectiveness of extant medical countermeasures prior to proceeding with particular studies, or establishing a more frequent schedule of DURC reviews to more closely monitor the research as it evolves. It is also expected that a communication plan will be established to ensure that DURC is communicated in a responsible manner. Federal funding agencies can provide advice and guidance on responsible communication, but recommendations on how to communicate research typically are not binding; ultimately, investigators and journal editors decide on how to communicate the research.

> **Analysis:** Some of the seven experimental effects within the scope of the DURC policies could be considered GOF studies. However, GOF studies that involve these effects are only subject to DURC oversight if they involve one of the 15 agents listed in the policies. Only two influenza viruses are within the scope of these policies; SARS and MERS coronaviruses are not. The DURC policies are also inherently subjective. While the list-based approach clearly delineates projects that are subject

[39] The definition of dual use research of concern listed in the *USG Policy for Oversight of Life Science DURC* (USG, March 2012) and the *USG Policy for Institutional Oversight of Life Sciences DURC* (USG, September 2014) is "Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."

[40] The policy for Federal DURC oversight requires Federal funding agencies to compile biannual inventories of projects identified as being subject to DURC oversight. As part of this process, Federal agencies have been identifying projects involving MERS and LPAI H7N9 influenza and proactively managing risks associated with those projects, as necessary.

to oversight, the definition of DURC, and to a lesser extent, the seven experimental effects, all require significant judgment and interpretation.

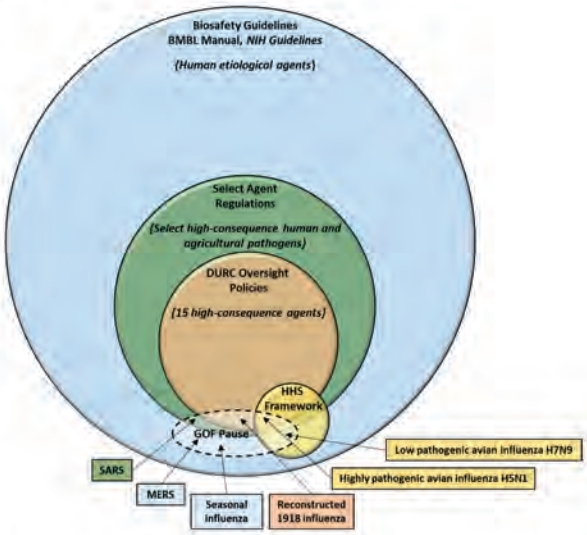

Figure 3. Comparison of the scope of different policies for the oversight of life sciences research involving pathogens. Oversight policies apply to research involving specified agents or procedures. GOF studies involving pathogens or manipulations covered under a given policy would be subject to oversight described by that policy.

Federal-Level Review of Certain Gain-of-Function Studies

The only U.S. policy that specifically addresses GOF studies is the *Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets (HHS Framework)*, issued by HHS in February, 2013. Under the *HHS Framework*[41,42] certain proposals with the potential for generating highly pathogenic avian influenza H5N1 viruses that are transmissible among mammals by respiratory droplets receive special review and

[41] *A Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets*. U.S. Department of Health and Human Services, February 2013. http://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

[42] Patterson, A., et al. *A Framework for Decisions about Research with HPAI H5N1 Viruses*. Science. 2013 Mar 1: 339(6123): 1036-1037.

approval before being funded by HHS. This policy was subsequently expanded to include review of similar proposals involving low pathogenic avian influenza H7N9 viruses.[43]

Funding agencies within HHS (including NIH, CDC, and FDA) review relevant proposals for risks and benefits, and refer relevant studies to a Department-level review group, the HHS HPAI H5N1 Gain-of-Function Review Group, for advice prior to funding the proposal. The review group includes a wide range of interdisciplinary expertise from across HHS, and can draw on additional experts within the U.S. government if necessary. HHS reviews GOF research proposals that are subject to the *HHS Framework* and makes recommendations to HHS funding agencies about whether the study is acceptable for funding and whether additional measures may be needed to mitigate risks. HHS considers a number of factors including the following criteria, which must be met in order for a GOF study to be acceptable to receive HHS funding:

1. The virus anticipated to be generated could be produced through a natural evolutionary process;
2. The research addresses a scientific question with high significance to public health;
3. There are no feasible alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach;
4. Biosafety risks to laboratory workers and the public can be sufficiently mitigated and managed;
5. Biosecurity risks can be sufficiently mitigated and managed;
6. The research information is anticipated to be broadly shared in order to realize its potential benefits to global health; and
7. The research will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research

Analysis: The *HHS Framework* requires an explicit consideration of the risks and benefits associated with certain GOF studies prior to making a funding decision. This allows HHS to identify potential risks prior to funding the research and make recommendations about risk mitigation—including consideration of alternative approaches or modifying the experimental design—at the outset. This review process also involves broader expertise including, ethical, legal, security, intelligence, and others. The criteria that must be met in order to receive funding are subject to judgment and interpretation. The scope of the *HHS Framework* is also quite narrow and currently covers only projects involving two influenza viruses and that involve one specific experimental outcome (mammalian transmission by respiratory droplets); other GOF studies involving different pathogens do not receive this pre-funding review.

Reviews under this framework are conducted by a group internal to the U.S. government. Reviewing GOF studies in a confidential setting allows for the examination of potentially sensitive scientific, proprietary, and personal information, and allows for discussions that may be sensitive from a national security or public health preparedness perspective. However, such reviews do not achieve the level of transparency desired by some stakeholders and also make it difficult to independently assess the effectiveness of the review process. Finally, the *HHS Framework* was in

[43] Jaffe, H.W., et al. *Extra Oversight for H7N9 Experiments*. Science. 2013 August 16: 341(6147):713-714.

place for less than two years when the October 2014 funding pause was enacted and only a handful of GOF projects have been reviewed to date, making it difficult to fully evaluate this policy's strengths and limitations.

In response to the funding pause[44], the National Institute for Allergy and Infectious Diseases (NIAID), within the NIH, developed a process for considering on a case-by-case basis studies that might be subject to the GOF pause. Reviews by NIAID include a detailed consideration of the science, including a specific examination of the viral strains in question and specific experiments being proposed. NIAID begins by consulting the investigators and an internal NIAID group determines whether the projects are subject to the pause. When identifying projects subject to the funding pause, NIAID used a fairly broad interpretation of the language set forth in the pause statement and paused, at least initially, more projects than were ultimately determined to meet the scope of the pause policy. NIAID also sought exceptions (using a mechanism provided for in the USG's moratorium statement) for projects that were deemed critical to public health or national security. In determining whether an exception to the pause might be warranted, NIAID considered the intent of the research, the availability of countermeasures, potential alternative approaches, the risks of not conducting the research, and the available mechanisms for ongoing oversight. Exceptions may only be granted by the NIH Director.

Analysis: NIAID's process for identifying GOF projects that are subject to the funding pause is rigorous and serves as an example of federal-level identification and review of GOF studies of potential concern. It includes extensive scientific review and is performed by individuals with experience reviewing projects for DURC potential. It does not involve the same expertise that is provided under *HHS Framework* reviews such as national security, ethics, or legal. Given the limited number of projects that have been examined by NIAID it is difficult to fully evaluate the effectiveness of this approach.

Sharing and Communicating Scientific Findings and Research Products

The majority of life sciences research is conducted in academic settings and the results are communicated openly in scientific journals and public forums. For a small subset of research with national security implications, there are policies in place to restrict access to scientific information or products. Under National Security Decision Directive (NSDD) 189, dissemination of fundamental research is to remain unrestricted to the maximum extent possible and in instances where restriction is necessary for national security, classification is to be the appropriate mechanism for restricting access.[45] Life sciences research that requires classification is classified at its outset and conducted in

[44] *United States Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses*. U.S. Government, October 17, 2014. http://www.phe.gov/s3/dualuse/documents/gain-of-function.pdf

[45] NSDD 189 (September 21, 1985) defines fundamental research as "basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons." https://research.archives.gov/id/6879779

designated facilities that are equipped with the infrastructure and personnel with appropriate level national security clearances to perform the research. Retroactively classifying research that was conducted in an unclassified setting is immensely challenging and may be unfeasible.

Export controls are federal regulations that regulate exports that have national security or foreign policy implications. Certain materials and information related to biological agents and genetic elements, vaccines, equipment, and related technologies are covered by export control regulations. Furthermore, the transfer of controlled information to a foreign national within the United States may be considered to be an export to that foreign national's country. The regulations are complex but, in general, they specify which items, when being shipped to which destinations, will require an export license. Life sciences research that is openly published is not subject to export controls, but information that is withheld from publication by the investigator or research institution based on security concerns may become subject to export control regulations, and an export license may be required before that information can be shared with foreign nationals. Most biological research activities that are subject to export controls fall under the Department of Commerce's Export Administration Regulations, which control items that have both military and civilian applications.[46] However, some might fall under the jurisdiction of the State Department's International Traffic in Arms Regulations.[47]

A number of scientific journals and families of journals have policies for identifying and reviewing manuscripts that raise biosecurity and biosafety concerns. These efforts are commendable but some have noted the challenges associated with trying to identify DURC or implement risk mitigation measures at the publication stage.[48, 49] NSABB has previously developed strategies and a risk assessment tool to assist in the development of a responsible communication plan for DURC, which might include altering the content, distribution, or timing of a publication.[50] The U.S. government has no authority to mandate redaction, restriction, or classification of a scientific publication that it does not own or control, and efforts to develop a mechanism for restricting communication of unclassified information to only those who require access, remain challenging and to date, unsuccessful.[51]

Analysis: Once a study has been completed, it is difficult to limit the distribution of or access to the findings, particularly if the study was conducted in an open, academic environment. Oversight of DURC, and in particular GOF studies involving pathogens with pandemic potential, may be most

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[46] Export Administration Regulations, 15 CFR Parts 730, 734, 736, 742, 744, and 745. https://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear

[47] International Traffic and Arms Regulations, 22 U.S.C. 2778 https://www.pmddtc.state.gov/regulations_laws/itar.html

[48] Casadevall, A., et al. *Dual-Use Research of Concern Review at American Society for Microbiology Journals*. mBio 6(4):e01236-15. 2015.

[49] Atlas, R., et al. Journal editors and authors group statement on scientific publication and security. Science, 299:1149. 2003.

[50] *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information*. National Science Advisory Board for Biosecurity, June 2007. http://osp.od.nih.gov/sites/default/files/resources/Framework%20for%20transmittal%20duplex%209-10-07.pdf

[51] Research information produced under a U.S. government grant is not considered to be owned or controlled by the Federal Government. However, under the Invention Secrecy Act, the U.S. government can nevertheless impose secrecy orders on patent applications if the publication or disclosure of the ensuing patent would be detrimental to national security.

feasible and effective if it occurs 1) upstream (i.e., during the review of proposed studies and before experiments are initiated) and 2) in an ongoing manner while the research is being conducted. Classification may be an option for certain GOF studies, but this would require these studies to be conducted in significantly different settings than they are currently. Further, although certain GOF studies have raised concerns about whether they should be published, it is unlikely that such manuscripts would meet the criteria for classification under U.S. government classification authorities. It is conceivable that certain studies should not be undertaken at all or not published because of unanticipated findings. However, it may be very difficult to predict at the proposal stage whether findings of concern might arise during the experiment, and unanticipated findings that raise concern may be unavoidable. Individual investigators or journal editors have, on security grounds, decided to redact certain material from publication, possibly triggering export controls on the redacted material, but in general such a redaction could not be mandated by the U.S. government.

Broader U.S. Biosafety and Biosecurity Efforts

Parallel to the GOF deliberative process, the USG also initiated broader reviews of biosafety and biosecurity policies and procedures following a series of laboratory incidents occurring at federal institutions in 2014. The Holdren-Monoco memorandum[52] called for federal and non-federal reviews to provide recommendations to strengthen the biosafety and biosecurity practices and oversight system for USG funded research. The memo outlined three immediate actions for U.S. government Departments and Agencies:

1. Conduct a comprehensive review of current biosafety and biosecurity protocols to ensure adequacy and appropriateness for today's infectious disease research
2. Inventory and document culture collections
3. Increase attentiveness throughout research community to ensure the safety of laboratory workers and the American public.

In September 2015, The White House National Security Council tasked the Federal Experts Security Advisory Panel (FESAP) to 1) identify needs and gaps and make recommendations to optimize biosafety, biosecurity, oversight, and inventory management and control for biological select agents and toxins (BSAT); 2) identify actions and any regulatory changes to improve biosafety and biosecurity; and 3) identify an approach to determine the appropriate number of high-containment U.S. laboratories required to possess, use, or transfer BSAT. To obtain broad stakeholder recommendations, the National Science and Technology Council established the Fast Track Action Committee on Select Agent Regulations (FTAC-SAR). In October 2015, USG released the FESAP and FTAC-SAR recommendations[53] that address: the culture of responsibility, oversight, outreach and education; applied biosafety

[52] August 2014 White House Memorandum – Enhancing Biosafety and Biosecurity in the United States https://www.whitehouse.gov/sites/default/files/microsites/ostp/enhancing_biosafety_and_biosecurity_19aug2014_final.pdf
[53] *Report of the Federal Experts Security Advisory panel.* December 2014. http://www.phe.gov/s3/Documents/fesap.pdf; *Fast Track Action Committee Report: Recommendations on the Select Agent Regulations Based on Broad Stakeholder Engagement.* http://www.phe.gov/s3/Documents/ftac-sar.pdf

research; incident reporting; material accountability; inspection processes; and regulatory changes and guidance to improve biosafety and biosecurity. The USG is implementing these recommendations.[54]

[54] *Implementation of Recommendations of the Federal Experts Security Advisory Panel and the Fast Track Action Committee on Select Agent Regulations*, October 2015. http://www.phe.gov/s3/Documents/fesap-ftac-ip.pdf

5. Findings of the NSABB

In developing its findings (Box 2), the NSABB considered the results of the RBA, policy analysis and decision-making frameworks, discussions of ethics, and perspectives of domestic and international stakeholders.

Box 2. Summary of Findings

Finding 1. There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight.

Finding 2. The U.S. government has several policies in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented.

Finding 3. Oversight policies vary in scope and applicability, and do not cover all potential GOFROC, therefore, current oversight is not sufficient for all GOF research of concern.

Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and that the benefits of the research are being fully realized.

Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted because the potential risks associated with the study are not justified by the potential benefits. Decisions about whether specific GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important and need to be taken into account.

Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires both federal and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security.

Finding 7. Funding and conducting GOF research of concern encompasses many issues that are international in nature.

Finding 1. There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF research—GOF research of concern (GOFROC)—entail risks that are potentially significant enough to warrant additional oversight. As with all life sciences research involving pathogens, GOF studies entail inherent biosafety and biosecurity risks. GOF research involving the generation of pathogens with pandemic potential involves the greatest risks. A laboratory accident involving such a pathogen could potentially release a pathogen that could spread rapidly and efficiently through the human population. A laboratory pathogen with enhanced characteristics could also, if malevolently used, pose a greater threat to national security or public health than similar misuse involving a wild type pathogen. The probability that such events would occur is low but non-zero and the potential consequences are uncertain but potentially significant.

Gryphon's biosafety risk assessment identified studies involving enhanced transmissibility, enhanced pathogenicity, and evasion of immunity as entailing the highest risks for coronaviruses, seasonal influenza, and avian influenza.[55] Manipulations that increase transmissibility, increase pathogenicity, and enable a pathogen to more readily spread through the population have the greatest potential to increase risk; in some strains even a moderate increase might be a concern.

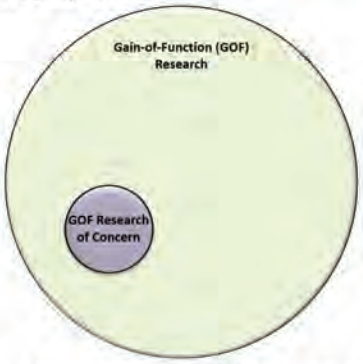

Figure 4. Conceptual categorization of GOF research involving human pathogens. GOF research includes a broad range of experimental approaches, most of which do not raise significant concerns. GOF research of concern represents a small subset of all GOF research that can be reasonably anticipated to result in generation of a pathogen with pandemic potential, described as a pathogen that is likely both highly transmissible and highly virulent in humans.

To help categorize studies based on the level of concern stemming from their associated risks, the NSABB has designated studies as: GOF research and GOF research of concern (GOFROC) (Figure 4). The term "GOF research" would encompass all studies whereby some characteristic of the pathogen is enhanced by experimental manipulation. The vast majority of GOF research does not raise any significant concerns; these studies do not entail novel or significant risks and are subject to appropriate oversight to manage risks. GOF research of concern, or GOFROC, represents the small subset of studies that result in the generation of a pathogen with pandemic potential—that is, a pathogen that is highly virulent and highly transmissible, as judged by its likely ability to spread among human populations (see Recommendation 1 for more thorough descriptions of these attributes).

[55] *Risk and Benefit Analysis of Gain-of-Function Research, Final Report*. Gryphon Scientific, April 2016. http://www.gryphonscientific.com/wp-content/uploads/2016/04/Risk-and-Benefit-Analysis-of-Gain-of-Function-Research-Final-Report.pdf

Finding 2. The U.S. government has several policies in place for identifying and managing risks associated with life sciences research. There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF research of concern could be implemented. Federally-funded life sciences research in the U.S. is conducted in accordance with occupational health and safety laws and regulations, the *NIH Guidelines*, the BMBL, policies for the federal and institutional oversight of DURC, the select agent regulations, export control regulations, international treaties and agreements, and other relevant policies. HHS has also developed a framework for guiding funding decisions for certain GOF studies involving H5N1 and H7N9 influenza viruses. Together, these policies aim to mitigate biosafety risks, biosecurity risks, and other risks associated with life sciences research, including many of the GOF studies that have raised concerns.

U.S. policies involve oversight and help manage risks during several phases throughout the research life cycle including the proposal review, the funding decision, during the conduct of the research, and at the time the research is being communicated. There are also numerous entities that are responsible for providing oversight, managing risks or issuing guidance, including funding agencies, federal advisory committees, institutional review and compliance committees, individual investigators, and journal editors.

While effective implementation of these policies can manage much of the risk associated with life sciences research, some GOFROC is more thoroughly monitored than others. Additionally, coverage under current policies is incomplete (e.g., GOFROC funded and conducted by/within the private sector may not be subject to federal oversight). Institutional oversight also varies. For example, IBCs differ in capabilities and expertise, and institutional resources and cultures vary. In addition, there is limited data describing the rate and extent of laboratory accidents, near misses, and security breaches. Little comprehensive data about these critical issues exist, and no entity is currently authorized to collect all of the desirable information that would inform risk-benefit assessments.

Finding 3. Oversight policies vary in scope and applicability, and do not cover all potential GOFROC, therefore, current oversight is not sufficient for all GOF research of concern. U.S. policies are applicable to some but not all GOFROC. Risks associated with GOFROC that does not involve select agents or pathogens subject to oversight under the USG DURC policies or the *HHS Framework*, would largely be managed at the institutional level, in accordance with guidance provided in the *NIH Guidelines* and BMBL. In general, GOFROC that is not conducted with USG funds is not subject to oversight by a federal funding agency.[56] Other countries also fund and conduct life sciences research, including GOF studies, which are beyond the purview of the U.S. government as well.

[56] Research involving a select agent, whose oversight is articulated in Federal statute and requires compliance from all researchers and institutions, would be subject to Federal oversight, regardless of the funding source. Some privately-funded research being conducted at institutions that receive Federal funding for that research may also be subject to oversight under the *NIH Guidelines*, USG DURC policies, or other policies.

In addition, the USG oversight policies vary. Different policies are aimed at managing different risks, and each is implemented by various Federal Departments and Agencies. This can result in redundancies as well as gaps in oversight, as the various policies are not sufficiently harmonized.

Finally, full compliance with policies is essential to their effectiveness. The effectiveness of policies can be enhanced by a commitment to proper implementation and enforcement at the federal, institutional, and individual investigator levels. This can include training, education, codes of conduct, and other mechanisms for continuing to build a culture of responsibility.

Finding 4. An adaptive policy approach is a desirable way to ensure that oversight and risk mitigation measures remain commensurate with the risks associated with the research and that the benefits of the research are being fully realized. Many, but not all, of the policies that apply to GOF studies are adaptive in nature. The BMBL is updated periodically. The *NIH Guidelines* and the select agent programs are updated or revised periodically as well and both have processes for seeking external advice for informing policy development. The DURC policies and the *HHS Framework* do not have articulated mechanisms for reviewing or updating the policies, or for seeking input on policy development (though both state an intention to be updated as necessary).

Great uncertainty is inherent in conducting risk-benefit assessments with currently available data and the uncertainty associated with several key parameters of the risk and benefit assessment made its interpretation challenging. Such uncertainty about risks and benefits may also make risk management difficult. An adaptive policy approach would facilitate refinement of GOF risk management as knowledge and experience are acquired.

Finding 5. There are life sciences research studies, including possibly some GOF research of concern, that should not be conducted because the potential risks associated with the study are not justified by the potential benefits. Decisions about whether specific GOFROC should be permitted will entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations, including legal, ethical, public health, and societal values are also important and need to be taken into account. Examples of studies that should not be conducted for ethical reasons include those that: involve human subjects who have not been provided and signed an informed consent document approved by an IRB; are anticipated to cause undue harm to a human subject; or that entail risks that are unjustifiable in the light of the benefits. For example, the development of biological weapons is unethical and has been banned by international treaty.[57]

[57] Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Signed at London, Moscow and Washington on 10 April 1972; entered into force on 26 March 1975. Depositaries: UK, US and Soviet governments. http://www.opbw.org/

There may be GOFROC that should not be funded on ethical grounds but it is difficult to identify or describe such studies based on general or hypothetical descriptions. An ethical evaluation of a research study would entail an evaluation of the risks and benefits, which requires a thorough understanding of the scientific details of the proposal, including its aims and any foreseeable adverse consequences. In addition, the scientific, public health, and national security landscape is dynamic. Public health needs change as new diseases emerge. Risks may arise or diminish based on the availability (or lack) of effective countermeasures. Benefits may become more or less likely to be realized based on other enabling factors, such as new scientific findings or technologies. Decisions to fund GOF studies must take into account these nuances in the risk-benefit landscape.

The NSABB did not seek to develop a list of studies that should not be conducted but rather sought to develop general principles that describe what is acceptable and not acceptable for funding. A principle-based approach to guiding funding decisions is adaptable and likely more effective. However, one example of a scientific study that should not be conducted might be the insertion of a virulence gene from an unrelated organism into the genome of a virus that is transmissible through the respiratory route, which would be highly unlikely to occur through natural recombination. This study, and others that involve the transfer of virulence genes between disparate microbes would appear to lack public health benefit, since the novel, laboratory-generated pathogen is unlikely to arise naturally and would therefore entail potentially significant and unnecessary risks.

Finding 6. Managing risks associated with GOF research of concern, like all life sciences research, requires both federal and institutional oversight, awareness and compliance, and a commitment by all stakeholders to safety and security. Biosafety and biosecurity risks associated with life sciences research are managed through engineering controls, laboratory practices, medical surveillance and support, appropriate training, and other interventions. However, GOFROC has the potential to generate strains with significant risks that may require additional oversight and containment mechanisms. Managing the risks associated with GOFROC in particular requires a commitment to safety and security at the federal and institutional level that includes a strong foundation of training and a demonstrated commitment to compliance by the research institution, and the individual investigators at the local level.

Finding 7. Funding and conducting GOF research of concern encompasses many issues that are international in nature. The potential risks and benefits associated with GOFROC are international in nature. Laboratory accidents and intentional misuse could have global consequences. The benefits of vaccine and other medical countermeasure development and disease surveillance also have important international implications. The research enterprise is international as well, and GOFROC is being conducted in a number of countries already. While USG funding policy regarding GOFROC only directly affects domestic and international research within the purview of the U.S. government, decisions made by the United States in this area can influence GOFROC oversight policies globally.

Notably, as highlighted during presentations at NSABB and National Academies meetings, GOF research and GOFROC are being conducted in a number of countries and a variety of oversight mechanisms at the national and regional level are in place. In addition, a number of countries and international scientific organizations have been considering issues related to biosafety, biosecurity, dual use research, and GOFROC.[58, 59, 60, 61, 62, 63] International perspectives are important to the development of U.S. policy in this area and global engagement is necessary to foster effective oversight mechanisms and an international culture of responsibility around research involving pathogens.

The U.S. government, often in concert with the NSABB, has been engaged with the international community for many years and continues to work with those governments and organizations actively considering GOFROC-related issues. Presentations to the NSABB, its working groups, and at the National Academies meetings provided perspectives about the activities of foreign governments, international organizations, researchers, and others, and greatly aided the NSABB during the development of this report.

[58] National Academies of Sciences, Engineering, and Medicine. 2016. *Gain of Function Research: Summary of the Second Symposium, March 10-11, 2016*. Washington, DC: The National Academies Press. doi: 10.17226/23484.
[59] *Gain of function: experimental applications relating to potentially pandemic pathogens*. European Academies Science Advisory Council, EASAC policy report 27, October 2015. http://www.easac.eu/
[60] *Summary report: Dual Use Research On Microbes: Biosafety, Biosecurity, Responsibility*. December 10 – 12, 2014, Herrenhausen Palace, Hanover, Germany. https://www.volkswagenstiftung.de/dualuseresearch
[61] *France-US Bilateral Workshop on Dual Use Research Issues: Summary Report*, February 11, 2016. U.S. Department of State.
[62] Draghia-Akli, Ruxandra, Director of the Health Directorate at the Research DG, European Commission, presentation to NSABB working group, July 23, 2015.
[63] Donker, Marianne, Ministry of Health, Welfare and Sport, Netherlands, presentation to NSABB working group, July 23, 2015.

6. Recommendations of the NSABB

Based on its analyses and findings, the NSABB developed the following recommendations (Box 3) to the U.S. government.

Box 3. Summary of Recommendations of the NSABB

Recommendation 1. Research proposals involving GOF research of concern entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the federal and institutional levels.

Recommendation 2. An advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOF research of concern.

Recommendation 3. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOF research of concern.

- **Recommendation 3.1.** The U.S. government should develop a system to collect and analyze data about laboratory safety incidents, near-misses, and security breaches as well as the effectiveness of mitigation measures to inform GOF research of concern policy development over time.
- **Recommendation 3.2.** The U.S. government should develop or facilitate the development of a system to collect and analyze data about Institutional Review Entity (IRE) challenges, decisions, and lessons learned to provide feedback to the IRE community and to inform policy development for GOF research of concern over time.

Recommendation 4. In general, oversight mechanisms for GOF research of concern should be incorporated into existing policy frameworks when possible.

Recommendation 5. The U.S. government should consider ways to ensure that all GOF research of concern conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOF research of concern.

Recommendation 7. The U.S. government should engage the international community in a dialogue about the oversight and responsible conduct of GOF research of concern.

Recommendation 1. Research proposals involving GOF research of concern entail significant potential risks and should receive an additional, multidisciplinary review, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the federal and institutional levels.

GOFROC entails the generation of pathogens—perhaps novel pathogens—with anticipated pandemic potential. The risks associated with such studies are uncertain but potentially significant. It is possible that generating a laboratory pathogen with pandemic potential introduces a risk of a pandemic, albeit a low probability risk, that did not exist before that pathogen was generated. Therefore, a new, pre-funding review and approval mechanism is warranted before such studies should be undertaken. The NSABB's proposed conceptual approach for guiding funding decisions about GOFROC entails identifying GOFROC and subjecting such studies to an additional pre-funding review and approval process. The attributes that describe GOFROC, the principles that should guide funding decisions for GOFROC, and the steps in a proposed review/approval process for GOFROC are described below.

Identifying GOF research of concern

GOFROC is research that can be reasonably anticipated to generate a pathogen with pandemic potential. Determining whether a proposed research project is likely to do so will entail uncertainty and will require scientific and other expert judgment.

To be considered GOFROC, the research must, in a single step or over the course of multiple manipulations, be reasonably anticipated to generate a pathogen with both of the following attributes:

i. **The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.** To be considered "highly transmissible" the pathogen must be judged to have the capacity for sustained secondary transmission among humans, particularly, but not exclusively, by the respiratory route. Such a determination might be informed by data describing human infections by naturally-circulating isolates of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human infections. To be considered "capable of wide and uncontrollable spread in human populations" it must be judged that there would be limited options for controlling the spread of the pathogen other than patient isolation or quarantine. Such a determination might be made, for instance, if humans lack population immunity to the resulting pathogen, if the pathogen would evade or suppress the human immune response, if the pathogen would be resistant to medical countermeasures, or if existing countermeasures would be unavailable globally in sufficient quantities.

AND

ii. **The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.** To be considered "highly virulent" the pathogen must be judged to have the capacity for causing significant consequences in humans, such as severe disease and/or a high case fatality rate. Such a determination might be informed by data describing human infections by naturally-circulating strains of the pathogen or studies in relevant experimental mammalian models that serve as a proxy for human disease.

Any study involving the generation of a pathogen exhibiting the two attributes above would be considered GOFROC. However, it is generally anticipated that the following types of activities would not be considered GOFROC:

- Studies to characterize the virulence and transmission properties of circulating pathogens
- Surveillance activities, including sampling and sequencing
- Activities associated with developing and producing vaccines, such as generation of high-growth strains

Importantly, a proposed experiment need not involve the simultaneous enhancement of both phenotypes. Thus, research involving a naturally-occurring pathogen that exhibits one of the above attributes would be considered GOFROC if a study were anticipated to confer the second attribute to the agent (while retaining the first attribute). Other studies may generate a pathogen with the above attributes after a series of manipulations that enhance the phenotypes separately but ultimately result in a pathogen with both attributes. Any route of experimentation that is anticipated to ultimately generate a pathogen that exhibits both of the characteristics above would be considered GOFROC and should be reviewed carefully before it can be funded.

Appendix C describes examples of studies that would and would not be considered GOFROC. These examples are provided as general guidance. A more detailed consideration of the specific characteristics of a pathogen in question as well as the proposed experimental manipulations would be required to determine whether a research proposal is GOFROC.

Pre-funding review and approval of GOF research of concern

Proposals anticipated to involve GOFROC should be subject to additional review prior to making a funding decision and to substantial federal oversight throughout the course of the research, if funded. The NSABB developed principles that should guide the review and funding of these proposals. There should be a high degree of confidence that a study will be conducted in accordance with these principles before determining that the proposal is suitable for funding. Studies that cannot be or are not anticipated to be conducted in accordance with the principles below should not be funded.

Principles for guiding review and funding decisions

Only projects that are in line with **all of the following principles** should be considered acceptable for funding. The principles below are intended to embody the substantive ethical values described in section 4.2 and the process of applying these principles would involve scientific, security, ethical, and other considerations.

i. **The research proposal has been evaluated by a peer-review process and determined to be scientifically meritorious, with high impact on the research field(s) involved.** If GOFROC is to be funded and conducted it must first and foremost address a valuable scientific question or public health need.

ii. **The pathogen that is anticipated to be generated must be judged, based on scientific evidence, to be able to arise by natural processes.** It is difficult to predict the types of pathogens that can or will emerge in nature. Nevertheless, before a pathogen with pandemic potential is generated through laboratory manipulations it is essential to consider whether such a pathogen could arise in nature. GOFROC may be permissible if the study were to generate a pathogen that is anticipated to arise in nature or if the study were to provide insight into natural evolutionary processes. GOFROC would not be permissible if it were to generate a laboratory pathogen that is highly unlikely to arise in nature.

iii. **An assessment of the overall potential risks and benefits associated with the project determines that the potential risks as compared to the potential benefits to society are justified**. Prior to funding GOFROC, the anticipated risks and potential benefits must be carefully evaluated. In general, the potential benefits associated with a research project should be commensurate with or exceed the presumed risks. Projects involving significant risks and little anticipated benefits are ethically unacceptable and should not be funded. If the potential risks appear high, the possible benefits should also appear high. Risks should be managed and should be mitigated whenever possible. The extent to which risks can be mitigated should factor into the assessment.

iv. **There are no feasible, equally efficacious alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach.** Alternative approaches must be explored and critically examined before funding GOFROC. It is possible that the proposed experimental approach that raises concern is the only feasible approach for addressing the scientific question at hand. In other cases, modifications of the experimental design, use of attenuated or other strains that pose fewer risks to humans, or different approaches with less risk that may provide the same information may be feasible. Lines of experimentation that entail less risk should be pursued whenever possible.

v. **The investigator and institution proposing the research have the demonstrated capacity and commitment to conduct it safely and securely, and have the ability to respond rapidly and adequately to laboratory accidents and security breaches.** Prior to funding, the risks associated with proposed GOFROC must be identified and assessed, and clear, realistic plans for managing risks should be developed. In order to manage risks associated with GOFROC, an institution must have adequate facilities, resources, security, trained personnel, administrative structures, ongoing occupational health and safety monitoring procedures,

relationships with local public health authorities and first responders, and the ability to adapt to unanticipated situations by increasing containment or adding additional safety or security features. In addition to adhering to standards of compliance, an institution (and the investigators proposing the study) should have a demonstrated commitment to laboratory safety and security, scientific integrity, and the responsible conduct of research. The researchers and institution should be committed to a culture of responsibility, perhaps demonstrated through adherence to a formal code of conduct or other measures.

vi. **The results of the research are anticipated to be broadly shared in compliance with applicable laws and regulations in order to realize their potential benefits to global health.** Prior to funding GOFROC, consideration should be given to the type of research-related information and products that are likely to be generated. The research-related information and products are expected to be shared appropriately and a responsible communication plan should be developed at the outset, as appropriate. NSABB[64] and the U.S. government[65] have issued guidance for developing communication plans for dual use research of concern that include consideration of the content, timing, and distribution of the research information.

vii. **The research will be supported through funding mechanisms that allow for appropriate management of risks and ongoing federal and institutional oversight of all aspects of the research throughout the course of the project.** GOFROC should be funded through mechanisms that ensure appropriate biocontainment conditions are utilized, adequate biosecurity precautions are in place, and that the data and materials generated will be shared appropriately. The funding mechanism should allow for modification of required mitigation and oversight features, as well as research objectives during the course of the research, if needed.

viii. **The proposed research is ethically justifiable.** Determinations of whether proposed GOFROC should be undertaken involve value judgments to assess whether any potential risks are justified. Non-maleficence, beneficence, justice, respect for persons, scientific freedom, and responsible stewardship are among the values that should be considered when ultimately making decisions about whether to fund GOFROC.

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[64] Appendix 5, *Proposed Framework for the Oversight of Dual Use Research Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information*. National Science Advisory Board for Biosecurity, June 2007.

[65] Section E, *Tools for the Identification, Assessment, Management, and Responsible Communication of Dual Use Research of Concern: A Companion Guide to the United States Government Policies for Oversight of Life Sciences Dual Use Research of Concern.* U.S. government, September 2014.

The Review Process for Proposals Involving GOF Research of Concern

The NSABB proposes the following conceptual approach for guiding funding decisions about GOFROC (Figure 5). Review of research projects that may involve GOFROC would involve five steps:

1. Investigators and research institutions identify proposed GOFROC, as described by the two attributes for identifying GOFROC.
2. Funding agencies identify or confirm proposed GOFROC.
3. A Department-level panel of U.S. government experts reviews proposals involving GOFROC to determine whether the proposal meets the 8 principles for guiding funding decisions and to make recommendations as to whether the proposed research is acceptable for funding.
4. Funding agencies make a funding decision, and if the proposal is funded, establish risk mitigation plans and issue the funding award with appropriate terms and conditions to help ensure ongoing oversight.
5. Investigators and institutions conduct the research in accordance with any applicable federal, state, and local oversight policies and employ any necessary additional mitigation strategies. Federal agencies provide oversight to ensure adherence to established risk mitigation plans and funding terms.

Investigators and institutions identify GOFROC (Step 1). Prior to submission of an application for funding, investigators and research institutions should identify possible GOFROC and submit with the research proposal any relevant information such as plans for biosafety, biosecurity, and coordination with local and/or state public health and safety officials in the event of an accident or theft; descriptions of facilities available; a justification for the proposed approach that considers possible non-GOFROC alternatives that have been considered; and a discussion of the value and potential benefits of the proposed research. Identification of possible GOFROC should not affect a subsequent federal scientific merit review either positively or negatively.

> **A need for guidance to investigators and institutions.** The U.S. government should develop a "Points to Consider" document to provide guidance to investigators and institutions when preparing research proposals that may involve GOFROC. Such a document would describe any requirements for proposals involving GOFROC and provide guidance on the type of information that should be included in a proposal to facilitate its review. This document should be reviewed and updated as necessary.

Agency and Department-level review of GOFROC (Step 2 & 3). After the standard funding agency scientific merit review process, proposals that are determined to be scientifically meritorious and likely to be favorably considered for funding would also be reviewed by the funding agency (Step 2) to determine if they constitute GOFROC. Prior to being determined acceptable for funding, proposals identified by a funding agency as involving GOFROC would require an additional, higher level, Departmental review (Step 3). If a proposal does not involve GOFROC, it would proceed along the normal pathway for further evaluation and funding decisions.

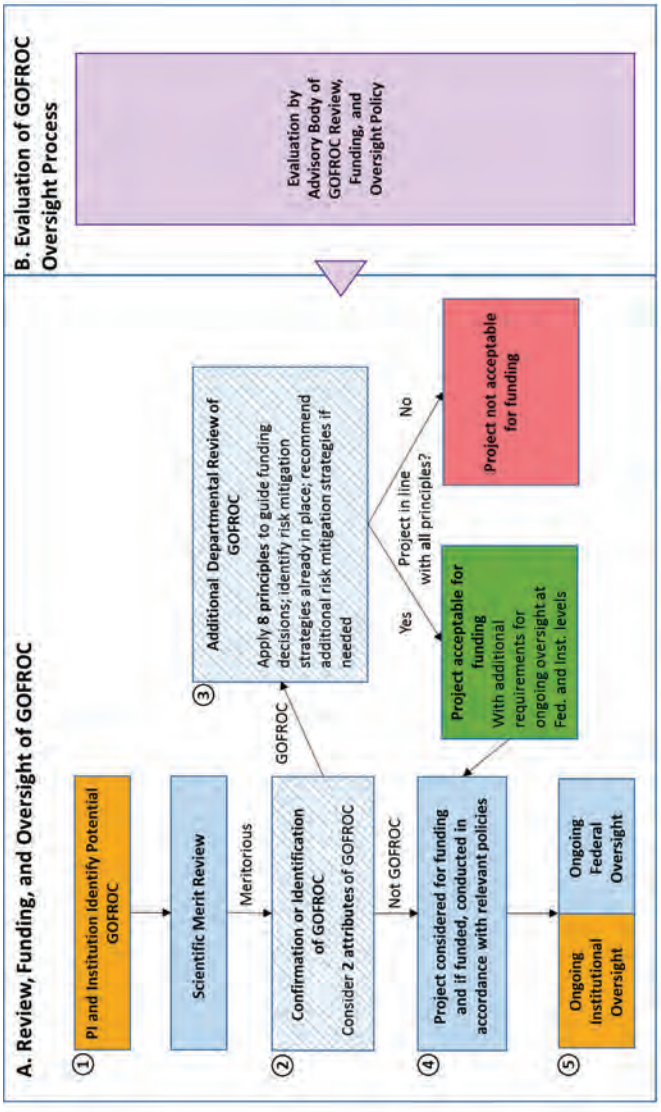

Figure 5. Proposed approach for the oversight of GOF research of concern. A) A conceptual approach for the identification, review, funding, and ongoing oversight of GOF research of concern. B) A Federally-appointed advisory committee would periodically evaluate the policies and processes developed for funding and providing oversight for GOFROC.

The additional review of proposals involving GOFROC would determine whether the proposed research aligns with the 8 principles to guide funding decisions. Applying these principles will help to ensure that the GOFROC is scientifically and ethically acceptable, that the risk-benefit balance is favorable, that alternative approaches are explicitly considered, and that the research can be performed safely and securely. It is envisioned that the additional review of proposals involving GOFROC would involve diverse, multidisciplinary expertise including scientific, public health, biosafety, national security and intelligence, legal, bioethics, and other perspectives. To the extent possible, the Departmental review process should be efficient, well-documented, and adaptive. In addition, the process should be structured to avoid real or apparent conflicts of interest and to provide consistency across USG agencies that might fund GOFROC. It is also envisioned that research institutions proposing the GOFROC might be asked for, and would have an opportunity to provide, any additional information that might be necessary for a thorough and substantive review of the research proposal. The NSABB also recommends (see Recommendation 2) that an advisory body that is designed for transparency play a role in the evaluation of the oversight policies for GOFROC.

Funding decision and risk mitigation (Step 4). During the course of the Department-level review, the relevant risk management plans should be critically evaluated and additional risk mitigation measures may be recommended in order for GOFROC to be considered acceptable for funding. A satisfactory risk management plan would entail appropriate biocontainment facilities and biosafety practices, appropriate standard operating procedures and administrative controls, occupational health and safety programs and security systems for protecting laboratory strains and reagents, and promoting personnel reliability. Some or all of the additional risk mitigation measures listed in Box 4 may also be recommended. Section 6.3 in Gryphon's RBA report also describe additional safety measures that many laboratories performing GOFROC have employed. These and a variety of additional measures could be required as conditions of funding.

Box 4. Additional risk mitigation measures to be employed, as appropriate, for GOF research of concern.

Risk mitigation features that should be considered prior to funding GOFROC include requirements to:

- Provide additional training to researchers
- Enhance biosafety practices or features, as warranted given the specific strains and proposed manipulations
- Enhance security measures around strains, reagents, notebooks, and personnel
- Prohibit certain additional GOFROC experiments without prior approval
- Treat the research as if subject to the USG DURC policies, if it is not already
- Conduct more frequent institutional biosafety and biosecurity reviews of the research
- Require more frequent progress reports and discussions with federal funding agency staff, particularly about unanticipated results that may raise concerns
- Conduct periodic site inspections/evaluations, if not already required
- Identify certain experimental outcomes that would trigger a re-evaluation of the risks and benefits prior to proceeding with a study
- Develop a responsible communication plan, specifically, including a description of biosafety and biosecurity practices that were employed for the research
- Communicate regularly and coordinate with federal, state, and local public health and safety officials on accident and theft response
- Conduct bioethics consultations at the local and federal level throughout the life cycle of the research
- Develop and/or adhere to an appropriate code of conduct

Ongoing oversight (Step 5). Finally, throughout the course of the funding, both federal and institutional oversight are critically important. The project should be carefully monitored to ensure that required conditions are met, that the principles guiding the decision to fund are still satisfied, and that any changes, significant developments, and publication/communication plans are discussed and addressed in a timely manner.

Recommendation 2. An advisory body that is designed for transparency and public engagement should be utilized as part of the U.S. government's ongoing evaluation of oversight policies for GOF research of concern. An advisory mechanism, such as a committee governed by the Federal Advisory

Committee Act[66], would allow for an independent examination of the U.S. government's policies for reviewing, funding, and conducting GOFROC (Figure 5.B.). Such a group could evaluate the additional review and funding processes for GOFROC to understand how decisions were made, identify challenges to implementing the policy, and provide recommendations, as needed. Importantly, this mechanism would also provide transparency, promote public engagement, and would facilitate continued dialogue about GOFROC.

Recommendation 3. The U.S. government should pursue an adaptive policy approach to help ensure that oversight remains commensurate with the risks associated with the GOF research of concern. The risk/benefit profile for GOFROC may change over time and should be re-evaluated periodically to ensure that the risks associated with such research are adequately managed and the benefits are being realized. An adaptive approach to the oversight of GOFROC would entail the continual evaluation of the risks and benefits associated with the research, as well as the burdens and effectiveness of the additional proposal review process and ongoing oversight measures. An adaptive approach would allow policymakers to learn from experience and update policies accordingly as the risk/benefit landscape changes. For instance, the risks associated with a research proposal or project may change if newly developed countermeasures become available or if new information emerges to clarify certain risks or enable certain benefits.

Recommendation 3.1. The U.S. government should develop a system to collect and analyze data about laboratory safety incidents, near-misses, and security breaches as well as the effectiveness of mitigation measures to inform GOF research of concern policy development over time. Examining such data would provide a better understanding of the risks, inform future risk assessments, and allow for the refinement of oversight policies over time.

Recommendation 3.2. The U.S. government should develop or facilitate the development of a system to collect and analyze data about Institutional Review Entity (IRE) challenges, decisions, and lessons learned to provide feedback to the IRE community and to inform policy development for GOF research of concern over time. Examining such data would provide a better understanding of the effectiveness and consistency of policy implementation and support local IRE decision-making.

Recommendation 4. In general, oversight mechanisms for GOF research of concern should be incorporated into existing policy frameworks when possible. Any additional oversight of GOFROC should be built into existing mechanisms rather than having the U.S. government develop a novel policy specific to GOFROC. Adapting or harmonizing current policies is preferable to developing entirely new oversight frameworks or wholly new approaches to manage the risks associated with these studies. There are precedents for additional Department-level pre-funding review of certain GOF studies (i.e.

[66] *Federal Advisory Committee Act*. http://www.gsa.gov/portal/content/100916

HHS Framework) as well as mechanisms for higher-level review and approval of certain studies (i.e., Major Actions, under the *NIH Guidelines*; restricted experiments, under the Select Agent Program). There are also mechanisms for continual Federal-level monitoring of biosafety and biosecurity risks for individual projects (i.e., USG Policy for Federal Oversight of DURC, select agent program) and established mechanisms for ongoing institutional oversight (i.e., IREs under the USG Policy for Institutional Oversight of Life Sciences DURC; IBCs under the *NIH Guidelines*). Wherever possible, these mechanisms should be employed to ensure the initial and ongoing oversight of GOFROC.

Importantly, not all GOFROC would necessarily be subject to the entire suite of U.S. oversight policies. For instance, some studies involving pathogens not included in the USG policies for DURC oversight or not on the select agent list could generate a pathogen with pandemic potential. Additional oversight measures may need to be stipulated at the time of funding for GOFROC proposals that are not subject to sufficient existing oversight. For instance, specific, enhanced containment practices may be required, or a project may require ongoing monitoring at the federal and institutional levels for its potential to be DURC. Box 4 describes a number of risk mitigation measures for GOFROC that could be implemented, potentially by leveraging existing policy frameworks.

Recommendation 5. The U.S. government should consider ways to ensure that all GOF research of concern conducted within the U.S. or by U.S. companies be subject to oversight, regardless of funding source. GOFROC conducted in the U. S. that is funded by the U.S. government or through private funding sources should be subject to equivalent oversight to ensure that the associated risks are adequately managed. The USG should consider ways to introduce oversight not only as a term and condition of a funding award but also via other mechanisms that would enable oversight of all relevant research activities, regardless of the funding source.

Recommendation 6. The U.S. government should undertake broad efforts to strengthen laboratory biosafety and biosecurity and, as part of these efforts, seek to raise awareness about the specific issues associated with GOF research of concern. Current discussions about GOFROC relate to broader domestic and international discussions about laboratory safety and security. A "top down" approach to managing the risks associated with GOFROC through federal policies and oversight is appropriate. However, top-down approaches alone, in the form of federal and/or institutional policies and leadership, will likely not be sufficient. It is also critical to have adequately trained personnel that value safe and secure laboratory environments for conducting GOFROC. Therefore, it will also be important to facilitate a "bottom up" approach whereby scientific leaders and professional societies, as well as research staff involved in the design and conduct of GOFROC, are educated about biosafety, biosecurity, and the responsible conduct of their research. The U.S. government should engage the research community with the goal of promoting a culture of responsibility, or "scientific citizenship," whereby all participants in the research enterprise have a sense of shared responsibility. Such a culture would

incorporate and stress the values of safety, security, compliance, and bioethics, and would work to promote public trust in the scientific enterprise.

Recommendation 7. The U.S. government should engage the international community in dialogue about the oversight and responsible conduct of GOF research of concern. Life sciences research is a global endeavor that continues to grow as more countries invest in their research capacities and as scientists move and collaborate across national boundaries. Life sciences research enables biomedical breakthroughs, pandemic preparedness, public health response efforts for emerging infectious diseases, and also provides an important economic driver. As more investigators undertake research involving pathogens, however, the associated risks become more likely to have international implications. The risks associated with GOFROC are notably international in nature since laboratory accidents or the deliberate misuse of pathogens with pandemic potential could have global consequences. Laboratories anywhere can undertake GOFROC, and publications in the open scientific literature may enable others to generate pathogens with pandemic potential.

NSABB has benefitted greatly from the extensive input into its deliberations by experts representing foreign governments, international organizations, academia, and others during presentations and comments at its meetings and the National Academies symposia.

The U.S. government should continue to engage the international community on issues related to dual use research, including policies, oversight mechanisms, science, research conduct, biosafety, biosecurity, containment, publication, funding, and bioethics. These issues are important in general and are particularly relevant to GOFROC. The U.S. government's international engagement efforts should seek to promote a global culture of responsibility and enhance the quality, legitimacy and effectiveness of oversight processes.

The U.S. government should build these efforts on the substantial international engagement activities that it and the NSABB have carried out since the NSABB was established. Such efforts have included three international roundtable meetings on dual use research issues, a series of webinars focusing on different global regions, and an international consultative workshop on GOF issues[57]. In addition, the U.S. National Academies and the European Academies Science Advisory Council have been engaged in the recent policy debates involving GOF studies and may be well positioned to continue international dialogue on the issue in coordination with national governments and relevant international organizations. The USG is encouraged to participate in such activities.

[57] Information about these meetings and activities, including agendas, summaries, and archived videocasts, can be found on the NSABB website at: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb/nsabb-meetings-and-conferences/international-engagement

7. Appendices

Appendix A. Description of NSABB Deliberations

NSABB Deliberations

The NSABB established two working groups to accomplish the two portions of its charge, which were to result in discrete work products.

- **Deliverable 1.** A report conveying NSABB's advice on the design, development, and conduct of the risk and benefit assessments.
- **Deliverable 2.** A report conveying NSABB's formal recommendations on the conceptual approach to the evaluation of proposed GOF studies.

DELIVERABLE 1: ADVISING ON THE RISK AND BENEFIT ASSESSMENTS

The first NSABB working group was tasked with advising on the design and conduct of the risk and benefit assessments. The group met between December 2014 and April 2015 and was consisted of 13 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies. The group convened by telephone conference calls and held a one-day in-person meeting.

The working group developed a draft *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, which was presented to the full NSABB and developed further based on input from all Board members. The final framework was approved by the full Board on May 5, 2015 and is included in this report as Appendix H. The recommendations in this framework were intended to inform the NIH as it guided the work of Gryphon Scientific in its risk and benefit assessments of GOF research. The aim of the NSABB's framework was to help generate risk and benefit assessments that would provide information that would allow the NSABB to make sound, evidence-based recommendations.

The NSABB recommended that the RBA focus on studies involving influenza viruses (seasonal strains, as well as high and low pathogenic avian strains) and SARS and MERS coronaviruses. Given that most pandemics are associated with respiratory transmission, pathogens capable of airborne transmission were considered to be of most acute concern. NSABB recognized that the RBA would provide information specific to the pathogens and scenarios that were examined, but intended that the assessments would generate information that could be more broadly interpreted and applied. Thus, NSABB's recommended approach to the assessments was intended to align with the USG's October 2014 statement, which states that while "gain-of-function studies that fall within the scope of research subject to the funding pause will be a starting point for deliberations, the suitability of other types of gain-of-function studies will be discussed."

DELIVERABLE 2: RECOMMENDATIONS ON A CONCEPTUAL APPROACH FOR EVALUATING PROPOSED GOF STUDIES

The second NSABB working group was tasked with developing draft recommendations on the conceptual approach for the evaluation of proposed GOF studies. The group met between June 2015 and May 2016 and consisted of 18 NSABB voting members as well as non-voting *ex officio* members and other *ad hoc* members from Federal agencies; (Appendix F). The group convened by telephone conference calls and met twice in person.

In addition to the working group's primary task of developing draft recommendations, it continued to provide input on the conduct of the risk and benefit assessments. The working group also received periodic status updates on the RBA from NIH and Gryphon, as well as reports on the commissioned ethics analysis by Dr. Michael Selgelid, examined draft work products, and reported back to the full NSABB.

In developing draft recommendations on a conceptual framework for evaluating proposed GOF studies, the working group structured its deliberations into three phases.

Phase I. Policy examination, research, and information gathering
Phase II. Interpretation, analysis, and synthesis of information and results
Phase III. Development of recommendations

In Phase I the working group sought to 1) identify and examine the information necessary to inform development of recommendations and 2) begin to identify principles that should guide the development of NSABB recommendations. The working group began its deliberations by considering the topic areas discussed at the NSABB meeting in May 2015, which included examination of relevant U.S. and international policy and consideration of broader perspectives such as those from funding agencies, national security experts, journal editors and scientific publishers, ethicists, and others. The working group held an in-person meeting to consult with experts on many of these topics. The working group also examined a number of published GOF studies and discussed how current policies might apply to such studies to provide oversight and risk mitigation.

During Phase II the working group focused on translating information about risks and benefits as well as ethics into decisions and recommendations. It examined how current policies apply to GOF studies and began to develop preliminary observations and findings. The working group discussed the ethical issues associated with funding and conducting GOF studies, particularly noting the values and ethical decision-frameworks that might be applied to policy decisions about GOF studies. The working group also developed analytic tools to assist it in systematically analyzing the results of the risk and benefit assessments. In November 2015, the working group began receiving briefings from Gryphon conveying the results of the RBA, as well as reports on ethics from Dr. Selgelid. The group sought to identify GOF

studies that might raise particular concerns and may require additional oversight or consideration prior to being funded.

In Phase III the working group developed its draft recommendations based on its analysis of the risk and benefit assessments and the ethics report, and consideration of all other information and perspectives that were examined.

Deliberations by the Full NSABB

The full NSABB convened times 6 times between October 2014 and May 2016. At these meetings the NSABB working groups provided progress updates and the full Board, deliberated the issues further, consulted with various experts, and sought public feedback. Public comments made at NSABB meetings and delivered to the NSABB in writing were carefully considered by the Board during its deliberations. The articles, resources, and stakeholders consulted by the NSABB and its working groups throughout this process are listed in Appendix E.

On November 25, 2014, NSABB voted to approve a statement conveying to the USG concerns it heard regarding the implementation of the funding pause for certain GOF studies.[68] On May 5, 2015, NSABB voted to approve its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*.[69] On May 24, 2016, NSABB voted to approve its *Recommendations for the Evaluation and Oversight of Proposed Gain-of-Function Research* (this document).

Role of the National Academies in the Deliberative Process

The National Academies played a critical role in the ongoing deliberative process. The National Research Council and the Institute of Medicine (now National Academy of Medicine) were asked to convene two forums to engage the life sciences community and to solicit feedback from scientists, the public, and other stakeholders. These forums involved discussion of principles important for the design of RBA of GOF research and of NSABB draft recommendations.

The first National Academies workshop was held on December 15 & 16, 2014 and focused on the potential risks and benefits associated with GOF studies, ways to assess risks and benefits, strengths and limitations of risk-benefit analyses, and the ethical and policy implications associated with funding and

[68] *Statement of the National Science Advisory Board for Biosecurity Regarding the USG Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS Viruses*. National Science Advisory Board for Biosecurity, November 25, 2014.
http://osp.od.nih.gov/sites/default/files/resources/Final%20NSABB%20Funding%20Pause%20Statement_12-12-14_0.pdf
[69] *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*. National Science Advisory Board for Biosecurity, May 5, 2015.
http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

conducting GOF studies that have raised concerns.[70] The discussions at this meeting directly informed the development of NSABB recommendations for conducting the RBA and its subsequent deliberations. In particular, the discussions about the potential risks and benefits associated with GOF studies informed NSABB's recommendations for the types of risks and benefits that should be analyzed by Gryphon. A common theme at this National Academies meeting was that the term "gain-of-function" is too broad and that in fact, only a subset of GOF studies truly raise concerns. NSABB applied this insight to its subsequent analysis of the RBA by seeking to identify the subset of GOF studies that raised significant or unique concerns. Finally, the legal and policy discussions that were initiated at this meeting prompted the NSABB to explore these topics, as well as ethical issues, further.

The second National Academies meeting was held on March 10 & 11, 2016 and included a discussion of the completed RBA and NSABB's preliminary findings and draft recommendations. NSABB's proposed attributes for identifying GOFROC were a major discussion point at this meeting, which resulted in NSABB refining and clarifying these attributes. In addition, there was significant discussion about the desirability of an adaptive policy approach, the need for data to inform policy decisions, and the role that a federal advisory committee might play in evaluating GOFROC or GOFROC policy. This meeting also had a significant focus on international issues and perspectives, with specific discussion of ongoing and potential future international activities in this area.[71]

The Risk and Benefit Assessments of GOF Studies

NIH commissioned Gryphon Scientific to perform formal risk and benefit assessments to provide the NSABB with qualitative and quantitative information about the risks and benefits associated with conducting certain GOF studies. Dr. Rocco Casagrande, the principal investigator for the study, presented to the NSABB on May 5, 2015 an overview of Gryphon's approach to conducting the RBA, which included a quantitative biosafety risk assessment, a semi-quantitative biosecurity risk assessment, and a qualitative benefit assessment. Prior to voting to finalize its *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research*, NSABB discussed with Dr. Casagrande its draft recommendations and how Gryphon's proposed approach aligned with NSABB's proposed recommendations. In June 2015, Dr. Casagrande presented and discussed a more detailed work plan with the NSABB working group. Over the course of the study, the NSABB working group received occasional progress reports from Gryphon and NIH staff, and were provided draft sections of the RBA as they became available. In November 2015 the NSABB working group began receiving the results of the completed RBA. A draft version of the report was posted in advance of the January 2016 NSABB meeting. Gryphon's final report was made publicly available in April, 2016.[72]

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[70] National Research Council and the Institute of Medicine of the National Academies. 2015. *Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop, December 15 & 16, 2014*. Washington, DC: The National Academies Press. doi: 10.17226/21666.

[71] National Academies of Sciences, Engineering, and Medicine. 2016. *Gain of Function Research: Summary of the Second Symposium, March 10-11, 2016*. Washington, DC: The National Academies Press. doi: 10.17226/23484.

[72] *Risk and Benefit Analysis of Gain-of-Function Research, Final Report*. Gryphon Scientific, April 2016. http://www.gryphonscientific.com/wp-content/uploads/2016/04/Risk-and-Benefit-Analysis-of-Gain-of-Function-Research-Final-Report.pdf

The NIH Office of Science Policy managed the contract with Gryphon Scientific. NIH staff met weekly with Gryphon to accomplish the goals of the Statement of Work and to ensure the recommendations provided in the NSABB's *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research* continued to inform the conduct of the RBA, as appropriate. NIH staff also consulted with NSABB *ex officio* members to get broader expertise and advice, and to help ensure that the risk and benefit assessments yielded information that would inform subsequent policy deliberations by the U.S. government.

Considering Ethical Issues Associated with GOF Studies

To guide the NSABB's evaluation of the risks and benefits associated with GOF studies and its development of recommendations, the Board sought additional input and analysis on ethics. NIH commissioned Dr. Michael Selgelid, Monash University, to examine the literature regarding the ethical issues associated with funding and conducting GOF research and to explore different ethical frameworks that might be utilized when considering how to evaluate the potential risks and benefits associated with GOF studies. Dr. Selgelid was also asked to provide an ethical decision-making framework that NSABB could consider using when analyzing the information provided in the risk and benefit assessments of GOF studies. The decision framework was to identify and consider ethical values that may not be fully captured by a RBA. Dr. Selgelid's analysis was to be accomplished in a neutral, objective manner, without making any definitive recommendations on whether and how to fund or conduct certain GOF studies or what policy course might be the most appropriate. Dr. Selegelid presented his initial work to the NSABB in September 2015 and delivered to the NIH a draft paper in December 2015, which was conveyed to the NSABB and made publicly available. A final version of the paper was made publicly available in April 2016.[73]

[73] Selgelid, M., *Gain-of-Function Research: Ethical Analysis*. April 2016. http://osp.od.nih.gov/sites/default/files/Gain_of_Function_Research_Ethical_Analysis.pdf

Appendix B. Summary of U.S. Policies for Biosecurity and Biosecurity Oversight

Oversight Measures	Risks Addressed	Description of Oversight	Analysis/Applicability to GOF Studies
Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition (December 2009) http://www.cdc.gov/biosafety/publications/bmbl5/index.htm	Biosafety risks	**Applies to:** Life sciences research involving infectious microorganisms or hazardous biological materials **Description:** General biosafety practices and biological containment for various classifications (risk groups) of microorganisms and etiological agents	BMBL does not describe GOF studies per se but does include summary statements and biocontainment guidance for research involving various influenza strains (including contemporary and non-contemporary human, high and low pathogenic avian, swine, the 1918 influenza strain, and reassortant viruses) and SARS-CoV. MERS-CoV had not emerged at the time of the last BMBL update but interim laboratory biosafety guidance was issued by CDC and is referenced by BMBL. BMBL is a guidance document and generally considered the authoritative reference for laboratory biosafety but it is not a regulatory document; compliance is voluntary.
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (November 2013) http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines	Biosafety risks	**Applies to:** Basic or clinical life sciences research that involves recombinant or synthetic nucleic acid molecules and is conducted at an institution receiving NIH funding for any such research **Description:** Describes roles and responsibilities of institutions and investigators in safely conducting research. Requires institutional review with a focus on the concepts of risk assessment, risk group classification of agents, physical and biological containment levels, practices, personal protective equipment, and occupational health. **Advised by:** NIH Recombinant DNA Advisory Committee (RAC)	The NIH Guidelines have been amended to include additional guidance for work with Risk Group 3 influenza viruses (1918 H1N1, H2N2, highly pathogenic avian influenza (HPAI) H5N1) to specify enhancements to biosafety level 3 containment, practices, and occupational health requirements. NIH Guidelines were amended again to require further enhancements to facilities, biosafety equipment and practices, including occupational health practices, for research involving HPAI H5N1 strains transmissible among mammals by respiratory droplets. NIH Guidelines are often used as a model of biosafety guidance by the broader scientific community but compliance is required only by institutions receiving such funding from the NIH. The scope is also limited to research involving recombinant or synthetic nucleic acids. Some IBCs also review and approve non-recombinant pathogen research; however, not all institutions require their IBCs to do so.
HHS and USDA Select Agent Program (as of July 2014) http://www.selectagents.gov/regulations.html	Biosecurity (physical and personnel) and biosafety risks	**Applies to:** Biological agents and toxins that have the potential to pose a severe threat to public health and safety, based on a set of criteria. **Description:** Regulates the possession, use, and transfer of select agents and toxins. Overseen by the Federal Select Agent Program. Requires registration of individuals and entities; federal background investigations; federal review of restricted experiments; training; institutional compliance; etc. **Advised by:** Intragovernmental Select Agents and Toxins Technical Advisory Committee	Studies that could be considered GOF studies, which involve pathogens on the select agent list, are subject to oversight by the SAP. Researchers and institutions performing such studies must receive favorable security risk assessments by the FBI, register with the SAP, receive training on the proper procedures and practices for handling such agents, and abide by other aspects of the regulations. SARS-CoV, HPAI H5N1 influenza, and 1918 influenza viruses are select agents and GOF studies involving these pathogens are subject to oversight by the SAP. Restricted experiments that would entail conferring antiviral resistance to these viruses would require additional review and approval prior to being conducted. GOF experiments involving MERS, and other agents not included on the select agent list, would not be subject to oversight by the SAP.

USG Policy for Federal Oversight of DURC (March 2012) http://www.phe.gov/s3/dualuse/Pages/USGOversightPolicy.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The federal DURC policy requires identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) by Federal funding agencies. DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
USG Policy for Institutional Oversight of DURC (September 2014) http://www.phe.gov/s3/dualuse/Pages/InstitutionalOversight.aspx	Biosecurity risks, particularly involving misuse of research information, products, and technologies (DURC)	**Applies to:** Life sciences research conducted at an institution receiving USG funding that involves any of 15 agents that pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects.	The institutional DURC policy requires federally-funded institutions to establish a system for the identification and oversight of certain pathogen research involving 7 experimental types, some of which can be described as GOF experiments (i.e., enhancing the harmful consequences of an agent; increase transmissibility; alter host range; etc.) DURC policies only apply to research involving 15 pathogens. Institutions may review other studies for DURC potential but are not required to do so. Certain GOF studies that involve other agents would not be subject to DURC oversight under the policies.
HHS Funding Framework for GOF studies (August 2013) http://www.phe.gov/s3/dualuse/Pages/HHSh5n1Framework.aspx	Biosafety and biosecurity risks associated with certain GOF experiments involving agents with pandemic potential	**Applies to:** Gain-of-function studies that are reasonably anticipated to generate HPAI H5N1 viruses that are transmissible, and LPAI H7N9 viruses that have increased transmissibility, between mammals by respiratory droplets **Description:** Describes an HHS Department-level review pre-funding review and approval process for certain GOF studies, which can result in funding, not funding, or funding with certain conditions and ongoing oversight.	The only policy focused specifically on funding decisions related to the types of GOF studies that have raised concerns. Narrowly focused only on specific GOF studies (enhancing mammalian transmissibility) on two avian influenza viruses; other GOF studies may raise concern and would not be reviewed under this framework.
USG Export Control Regulations http://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear		**Applies to:** Export or release of equipment, software and technology, chemicals, microorganisms, toxins, and other materials and information deemed dual use or strategically important to U.S. national security, economic, and/or foreign policy interests	Comprehensive set of federal regulations that control and restrict the export and release of sensitive equipment, software and technology; chemical, biological, and other materials and information as a means to promote national security interests and foreign policy objectives.

Appendix C. Identifying GOFROC: Examples of Studies that Would and Would Not be Considered GOFROC

Experiment that is anticipated to entail GOFROC and therefore require additional pre-funding review and approval	Rationale (See NSABB Rec. 1 for description of GOFROC Attributes)
An experiment that is anticipated to generate avian influenza viruses that are transmissible by the respiratory route in mammals, if the starting virus is highly virulent in humans.	**Attribute 1.** The experiment is anticipated to increase transmissibility by the respiratory route in a relevant experimental mammalian model. Further, altering the host range from birds to mammals could generate a virus to which there is no existing population immunity, resulting in a virus capable of wide and potentially uncontrollable spread among humans. **Attribute 2.** Since the starting virus is highly virulent in humans it can be reasonably anticipated that the resulting virus will remain highly virulent in humans.
Reassortant studies involving avian and human influenza virus strains conducted to identify reassortants with pandemic potential that could arise naturally.	**Attribute 1.** Given the starting viruses and the goal of the experiment to identify/select for reassortants that are potentially highly transmissible in mammals, it can be reasonably expected that one or more of the resulting strains could be highly transmissible in humans. Since the resulting viruses are reassortants between bird and human influenza viruses, it can be anticipated that the antigenicity of at least some will remain avian-specific such that human populations would not be expected to have been exposed to such a strain or have pre-existing immunity. Therefore, it can be anticipated that a resulting virus could be capable of wide and uncontrollable spread. **Attribute 2.** Whether or not any of the starting viruses are highly virulent in humans, it can be reasonably anticipated that the expression of novel combinations of gene segments, derived from different influenza strains, in reassortant viruses could result in a range of characteristics that includes high virulence.
Studies that would result in a strain of *Yersinia pestis* more likely to cause pneumonic forms of infection and be resistant to antibiotics.	**Attribute 1.** Given the ease of transmission of *Yersinia pestis* in previous pandemics, manipulations that would enhance its ability to spread by respiratory droplets and cause pneumonic infections would generate a highly transmissible pathogen. In addition, if this manipulation involved a strain that was resistant to frontline antibiotics, it can be anticipated that there would be limited options for controlling the spread of the pathogen among humans. **Attribute 2.** Since the starting agent is highly virulent in humans, particularly when spread through the respiratory route, it can be reasonably anticipated that the resulting agent will remain highly virulent in humans.

Experiment NOT anticipated to entail GOFROC and therefore not require additional pre-funding review and approval	Rationale
Studies aimed at generating a mouse-adapted MERS-CoV or other emerging human respiratory pathogen	**Not Attribute 1.** The starting virus is transmissible by the respiratory route among humans but is not highly transmissible. MERS-CoV transmission usually occurs as a result of close contact (e.g. providing unprotected care to an infected patient). Sustained community transmission has not been observed. Furthermore, the proposed adaptation to recapitulate human disease symptoms in mice would not be reasonably anticipated to enhance transmissibility thus the resulting virus would not be anticipated to be capable of wide and uncontrollable spread. **Possibly Attribute 2.** The starting virus is already highly virulent in humans and is associated with significant morbidity and mortality. However, it should also be noted that a mouse-adapted strain is likely to be less virulent in humans.
Studies enhancing the growth of seasonal influenza viruses, which may be performed during vaccine production	**Not Attribute 1.** The starting seasonal influenza virus is highly transmissible by the respiratory route in humans however, population immunity is likely to exist against circulating (and recently circulated) strains. Enhancement of growth is unlikely to result in a virus that can evade immunity, thus a virus capable of wide and uncontrollable spread would not be likely. **Possibly attribute 2.** Increasing seasonal virus' ability to replicate could potentially result in its increased ability to cause disease, which could result in highly virulent strains. **Note:** If this experiment were to involve an attenuated strain, as is often the case with vaccine production, it would be unlikely to result in a virus that is highly virulent in humans.
Antigenic drift studies whereby seasonal influenza viruses that are no longer neutralized by vaccine-induced immunity are generated and selected for in the laboratory.	**Not Attribute 1.** The starting seasonal influenza virus is highly transmissible by the respiratory route in humans. However, antigenic drift studies generate influenza viruses with some resistance to a specific immunization but do not change the antigenic character of the virus to a degree such that it would no longer be recognized by the human immune system. Given that the starting virus is a human virus—not one that naturally infects birds or other non-human hosts—there would likely be some pre-existing population immunity to the resulting strains. **Possibly attribute 2.** The experimental manipulation would not be anticipated to increase the virulence of the virus. The resulting strains are likely to exhibit a similar level of virulence as the starting strain. Whether its virulence is considered high or low would depend on the specific initial strain used.

Appendix D. Summaries of Stakeholder Perspectives

The NSABB consulted a wide range of experts and stakeholder groups including not only scientists and institutions that fund and conduct life sciences research, but a much larger and diverse array of groups including public health officials, medical practitioners, emergency responders, vaccine developers, scientific journals, as well as the general public, non-governmental organizations, individuals with international perspectives and others. To accomplish this, NSABB organized meetings with expert presentations and panels that offered opportunities for interested groups there and for individuals and organizations to express their views and contribute throughout the deliberative process in ways that have informed the NSABB deliberations. These include: several public full NSABB advisory committee meetings that included sessions dedicated to obtaining public comment, two public symposia hosted by the National Academies that obtained comments from the public at the meetings and online, as well as comments submitted to the NIH and NSABB by email, and discussions with subject matter experts during NSABB WG conference calls and in-person meetings. Also included below are views expressed in some of the articles that have been published on this topic. A complete list of the individuals consulted and articles examined by NSABB are listed in Appendix E. Gryphon also conducted extensive consultations with experts as part of their risk and benefit assessments of GOF research. Those experts are not listed here but a listing is available in Gryphon's report. [74]

The following is a synthesis of stakeholder ideas and opinions expressed during the deliberative process. Many of these points were conveyed in more than one venue and by more than one person or group.

Scientists and Others Favoring GOF Research

A variety of influenza and coronavirus researchers who conduct GOF research, and other life sciences researchers have stated that GOF studies are widely used and fundamental for understanding viruses, and therefore are crucial to undertake. This group generally favors conducting such research because it aims to benefit society. In their view, such research can be safely conducted under current oversight frameworks and further restrictions will impede valuable work that will lead to important scientific information about these viruses, leading to better drugs and vaccines, as well as to improving the specificity of surveillance, particularly for influenza. In addition, some GOF studies are viewed as essential, specifically those that alter host range or enhance pathogenicity in order to develop animal models of disease (for example, with SARS-CoV) or GOF studies that generate drug or countermeasure resistance, which are important in satisfying various FDA requirements for marketing approval. Those who support GOF studies also point out that such studies are needed for predicting what amino acid changes are important for human transmission and therefore are important for the selection of candidate vaccine viruses. They also argue that GOF studies are important for prioritizing viruses for risk management (surveillance) and that further work will make these applications more robust. These

[74] *Risk and Benefit Analysis of Gain-of-Function Research, Final Report*. Gryphon Scientific, April 2016. http://www.gryphonscientific.com/wp-content/uploads/2016/04/Risk-and-Benefit-Analysis-of-Gain-of-Function-Research-Final-Report.pdf

individuals also pointed to the risks associated with not doing GOF research (generally due to a lack of preparedness for natural public health threats) and argued that they must also be considered.

While acknowledging there are risks associated with GOF research, proponents believe those risks are manageable and have been overstated by some, as evidenced by the fact that laboratory acquired infections are rare and infections in the community as a result of releases from a laboratory are almost unknown. While risk cannot be zero, the work can be conducted safely and securely with appropriate risk mitigation including containment along with good training and with the implementation of robust occupational medicine programs. Alternatives to GOF do not always provide the full answer to key questions and may yield misinformation. Supporters of GOF studies have also expressed concerns about the effects of the current funding pause and possible additional oversight on the field of virology and young researchers, and feel that there are costs of not undertaking the work in question. A major need is for better definition of what is meant by GOF with a clear distinction between GOF studies and GOF studies of concern. Some have suggested that only viruses with increased transmissibility and pathogenicity represent risks that exceed those of other infectious diseases research. They have also noted that SARS and MERS viruses are different from influenza, and require a different risk assessment approach since they are already virulent human pathogens; GOF research is needed to develop animal models that will benefit development of countermeasures for coronaviruses. Some supporters have acknowledged that there may be some experiments that should not be done. Finally, proponents of GOF research have stated that the risks from naturally occurring influenza viruses, which they argue could be reduced through GOF work, are greater than risks from performing GOF studies.

Scientists and Others Critical of GOF Studies

Opponents and critics of GOF research have generally focused their concern on a subset of GOF studies—those that involve enhancing the pathogenicity and/or transmissibility in mammals (particularly by the respiratory route), which may result in the generation of novel pathogens with pandemic potential. Critics have argued that the generation of novel laboratory pathogens with pandemic potential poses major public health risks and some have argued such studies should not be conducted. They have presented and published calculations that suggest a high probability of global outbreaks of influenza that might kill hundreds of millions of people, as a result of the release from a laboratory of a novel GOF virus. There is some disagreement about these estimates and how likely a pandemic might be, but opponents generally argue that even a relatively low probability of a potentially massive outbreak with major consequences is unacceptable. Some critics of GOF studies have acknowledged that there are a number of GOF studies that can and should be conducted.

Opponents of certain GOF studies have also argued that the benefits of GOF studies have been overstated, or are questionable, and that the benefits generally do not outweigh the biosafety risks. They also question claims about the effectiveness of risk mitigation strategies, since human factors and human error are unavoidable and hard to control, and institutional compliance and competence may vary. Critics have disputed the value of GOF studies to surveillance stating that it is not possible to predict phenotype from genotype; therefore predicting the pandemic risk of newly emergent strains is

not achievable given the current state of knowledge. Also, in their view, controlling outbreaks doesn't require GOF research.

Opponents of GOF research tend to favor alternative types of research that, in their view, can provide the same public health benefits without the large risks. It was suggested that the approach should be on reducing the risk by reducing the hazard, as opposed to focusing on mitigation of the risk. For example, if a universal influenza vaccine was developed, the need for many GOF experiments would be eliminated. Critics want to see funds currently used for GOF work provided to other types of research, which would be a better use of scarce resources in their view. Overall, they view preventing major public health problems as paramount, and see a need to define a critical set of experiments that should not be done, or only be done with additional strong oversight. Opponents are also concerned about proliferation and other factors that may lead to misuse and biosecurity threats. Finally, opponents have pointed out a moral issue if risks and benefits of certain GOF studies are not fairly distributed globally.

Funding Agencies

Public and private funding agencies support GOF research that has raised concerns with the goal of improving public health and well-being. These organizations in the US and abroad are aware of the issues surrounding DURC/GOF studies and are working diligently to implement and comply with existing policies in their countries. Most funders have requirements and procedures in place as they apply policies and guidance to evaluate proposed work and to oversee funded work. Current approaches involve education and awareness campaigns, project risk evaluation, ethics reviews, development of risk mitigation plans, and post-award monitoring. Funders believe they can contribute to the GOF deliberative process as a result of their practical, on-the-ground experience with DURC and GOF. They are concerned that interpreting policy can be very challenging, since it requires considerable expertise and judgment. They would welcome workable policies with clear guidance and have noted some unintended consequences of the funding pause, which affected some GOF projects that had not raised particular concerns. Some foreign government funders view government funding as a poor control mechanisms because this does not cover privately funded research and research funded by other entities. National legislation, regulations, compliance, training, awareness-raising, and self-monitoring have been noted as important.

Biosecurity Experts and Others Concerned about National Security

The ultimate goal of national security professionals, as it pertains to life sciences research, is to protect public health from natural or man-made health threats. Those concerned with national security aim to prevent terrorists and others with malicious intent or misguided motives from using products or information from GOF research to cause harm. This may include deliberate release of pathogens into the community, targeting of researchers or research facilities, or interference with on-going research activities. GOF research represents biosecurity risks in addition to biosafety risks; these overlap but are different with regard to important legal, policy and regulatory issues. Managing biosafety risks may or may not also manage biosecurity risks; GOF policy must take both types of risk into account.

When trying to assess biosecurity threats, security professionals have noted the importance of avoiding assumptions and predictions about the motives and capabilities of those who might be planning biosecurity actions. Those in the security field gather a large variety of data, but often their information is imprecise and may require consideration of what is feasible and plausible. Because of the paucity of biosecurity events, it is very difficult to evaluate and predict the likelihood and consequences of a deliberate release or determine how to prevent and/or mitigate one, and different experts view this issue very differently. It was stated that research policy in itself is not be the appropriate solution to prevent specific biological threats but specific research policies could help raise awareness of security issues among researchers, which would be important.

Security and intelligence professionals have described the challenges associated with using classification as a potential risk mitigation strategy. Classification would effectively restrict access to sensitive research information and research products and would limit the number of laboratories able to perform the studies. This could be described as both a strength and a limitation, depending on one's perspective. Life sciences research that requires classification is typically classified at the outset; the retroactive classification of research that had been conducted in an open, academic setting is exceedingly difficult.

Scientific and Medical Journals

Scientific and medical journals have been at the forefront of the GOF issue. While a number of journals and families of journals have procedures in place for identifying DURC, including GOF and other biosecurity concerns in submitted manuscripts, many journal editors are not entirely comfortable with their role. Their mission is to transmit scientific information, not control it, and they may not have the security expertise or the access to such expertise to make the necessary judgments and decisions about risks associated with communicating certain research findings. Rejection and redaction are the major tools journals have to control dissemination of dual use information, and neither may actually address the concerns; they are also impractical to implement effectively. One suggestion voiced was to require that a description of the steps that were taken during conduct of the research to ensure safety be included in all manuscripts. Some journal editors and staff expressed a desire to get help in evaluating risks and mitigation strategies from an independent national group such as the NSABB and to involve them earlier in the overall process. Most think the publication stage is not the best point to exercise control or prevent misuse of data from GOF studies but realize they are the final gatekeepers. Earlier identification of DURC/GOF along with risk mitigation earlier in the research life cycle would reduce the burden on them. Also, new technology and novel publication venues make controlling information increasingly difficult, and, as noted above, not all journals are able to or choose to impose a rigorous review of manuscripts.

Countermeasure Developers

Companies and others that are attempting to develop vaccines and drugs against pathogens were represented in several discussions. Medical countermeasure (MCM) developers expressed quite divergent views and opinions. Those favoring GOF research argued that such work is absolutely

necessary for antiviral drug development because GOF experiments to select for drug resistant mutants as well as to develop animal models are part of the critical path to marketing approval. In their view, GOF studies also have had a major influence on developing influenza vaccines, both seasonal and pandemic, and are likely to result in improved ways to make even better vaccines in the future. GOF experiments are required for selection of strains with better growth properties, with key mutations that alter important phenotypes needed in the vaccine strain, and with incorporating characteristics of strains that are likely to emerge into proven backbones. It was noted that GOF studies that enhance virulence can help inform vaccine designers about which mutations to avoid incorporating into vaccine strains. This group is concerned that their efforts to improve public health may be limited or impeded by new policies and urge careful consideration of their needs as decisions are made.

Conversely, other MCM developers expressed the view that vaccine production now is little dependent on GOF research and that any possible benefits will be far into the future, although some feel long-term potential is there. Those who criticize GOF studies on these grounds have argued that vaccines are developed in response to strains that emerge as threats, rather than preemptively based on strains that might be predicted as threats. Rather than supporting GOF studies to enhance vaccine production and drug development, it has been suggested that the other constraints that impede MCM development be addressed, such as streamlining FDA approval procedures and improving manufacturing processes, which would have a much greater impact. These critics suggest limiting current GOF-related efforts and focusing attention and resources in other directions. Overall, they believe that impact of GOF research on vaccine and drug development has been overstated, and that the benefits articulated are more theoretical than practical.

The General Public and Organizations Representing their Views.

A number of stakeholders stressed the importance of having meaningful public engagement with input and participation as part of the deliberative process. It is important that communities that might be affected by accidents or the misuse of research have a say in the research that is being conducted, however, but this may not generally be the case in their view. Real transparency, with the public good as the foremost consideration, must be part of a truly independent decision-making process. They note that it is important to maintain public trust in the scientific enterprise by involving non-scientists at stages when their views can still have an impact on policy-making. Public opinion of science is harmed when decisions that influence public health and safety are made without such input or the input has no real impact. Conversely, effective community engagement can convert sceptics to supporters. More than one participant raised the concern that if risks and benefits are not equitably distributed, it is a serious ethical issue[75].

Other issues that were mentioned include: how harms will be compensated if a laboratory incident were to affect the surrounding community; the need for enough resources to conduct research safely; and the opportunity to learn from other industries such as the nuclear industry.

[75] The ethical issues are discussed in more depth elsewhere, notably, Dr. Michael Selgelid's ethical analysis and the section of this report on Ethical Values and Decision-Making Frameworks.

Research Institutions

Representatives of universities and other research institutions generally noted that there is already significant oversight of DURC and GOF at both the Federal and institutional levels. Biosafety professionals noted that potentially high risk projects would receive thorough scientific review and risk assessment, resulting in the development of risk mitigation plans, and on-going monitoring as a result of policies and requirements that are already in place. They cited concerns over any increase in compliance that would impose burdens on their already limited resources or impede researchers from doing valuable work. They have difficulty, at times, deciding what is DURC when reviewing specific projects and would welcome more specificity and guidance. Many emphasized the need for policies that are unambiguous and straightforward to implement.

Public Health Officials

Public health officials have expressed diverse opinions. Some believe that GOF research has and can continue to improve surveillance efforts, as well as vaccine and therapeutic development. Others expressed concerns that an accident involving a laboratory pathogen for which there are no countermeasures would be very concerning and difficult to respond to. At the local level it is important to have public health involvement in the decision-making process because they will be incident responders. Strong connections with state and local laboratories should be established for sharing information and might include involving them in the review process. It was also noted that GOF and related policies may impact sample sharing and impede international relations relating to public health efforts.

International Perspectives

A number of participants noted that there is much interest in the GOF/DURC issue internationally, and the international community is looking to see what the USG will do as a result of the deliberative process. It was noted that U.S. policy often influences policies globally and the international ramifications should be considered. Recent biosafety incidents in U.S. Federal labs have raised concerns among many in other countries about the ability of the U.S. to adequately manage risks. A number of countries have well-developed systems of policy and regulation that would address many or some GOF and DURC issues, though international policy approaches are generally somewhat different from those in the U.S. International experiences, activities, and perspectives were cited as important to consider in the deliberative process. A collaborative approach and active attempts to engage the international community was viewed as the most effective way to benefit all. Many favored launching an international dialogue soon, with development of broad concepts and points of agreement that could be shared by all, while still respecting national differences. In addition, it was suggested that academies of science and multi-national organizations such as the World Health Organization can play an important role in such interactions at the right time. Those with a particular interest in the international aspects of GOF research also cited ethical issues associated with the unequal distribution of risks and benefits

across rich and poor countries. It was noted that the European Commission uses a comprehensive ethics process for screening and monitoring DURC/GOF in research projects.[76]

Those with an Interest in the Deliberative Process Itself

A broad group of individuals offered comments on the deliberative process itself. This included: federal government personnel, ethicists, decision-making experts, policy experts, other scientists, and includes people who are also members of the previously-mentioned groups. Those concerned with the deliberative process generally called for a well-planned and executed, thorough, scientifically rigorous, and impartial RBA that is technically sound and socially acceptable. They favored a democratic deliberative process and a policy that incorporates decisions made by neutral parties. Policy should be created using risk-based and value-based approaches to achieve desired outcomes. They want the final policy resulting from the deliberative process to be capable of reasonably identifying and mitigating risks related to GOF while protecting scientific autonomy, research progress, discovery and innovation, public health, national security, and other critical interests.

Many see an adaptive process as desirable, and recommend collecting appropriate data about laboratory accidents and mitigation effectiveness. It was noted that risks and benefits will change as science advances. The funding decision-making process should be accountable and limit inherent conflicts of interest; the individuals or entities that make decisions is critical. Most favor using existing policies as the basis of policy for GOF, while acknowledging that current frameworks are not entirely adequate. The question of how to incorporate non-USG funded research into an acceptable framework was raised several times. Deciding how to decide is a key point.

Both proponents and critics of GOF studies criticized the term "gain-of-function" as being too broad and not descriptive enough. There was much discussion about the appropriate definition of GOF research of concern; many strong, often conflicting, views were expressed. Unfortunately, while it is important to have a working definition and criteria for what is GOF of concern as opposed to GOF, a binary distinction needed for deciding what requires extra scrutiny, GOF experiments are actually a continuum of increasing risk.

The funding pause was criticized for being too broad, and some described it as disruptive to scientific process. Finally, some feel that a definitive quantitative risk assessment is not possible because of the very large uncertainties and lack of critical information associated with doing such studies, and they question the value of any studies that are done.

[76] The EU Framework Programme for Research and Innovation, Horizon 2020. Guidance - *How to complete your ethics self-assessment*, version 1.0, 11 July 2014. http://ec.europa.eu/research/participants/data/ref/h2020/call_ptef/pt/h2020-call-pt-ria-ia_en.pdf#page=27

Appendix E. Consultations, Comments, and Sources Considered During NSABB Deliberations

Table 1A. Invited speakers, presenters, and panelists. This table lists invited individuals who presented at NSABB, NSABB working group, and the National Academies meetings. Members of the NSABB or an NSABB working group are listed if they presented as a subject matter expert on a specific topic.

Speaker/Commenter	Affiliation/Location	Venue
Regine Aalders, M.Sc.	Embassy of the Netherlands, Washington, D.C.	NSABB Full Board Meeting (January 7-8, 2016)
Nisreen AL-Hmoud, Ph.D, M.Phil.	Royal Scientific Society of Jordan	National Academies Workshop (March 10-11, 2016)
Ronald Atlas, Ph.D.	University of Louisville	National Academies Workshop (December 15, 2014)
Ralph Baric, Ph.D.	University of North Carolina at Chapel Hill	National Academies Workshop (December 15, 2014)
Kavita Berger, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015), In-person WG Meeting (November 9, 2015)
Thomas Briese, Ph.D.	Columbia University	National Academies Workshop (December 15, 2014)
Michael Callahan, M.D., D.T.M.&H., M.S.P.H.	Massachusetts General Hospital; Harvard Medical School	National Academies Workshop (March 10-11, 2016)
Arturo Casadevall, M.D., Ph.D.	Johns Hopkins Bloomberg School of Public Health; mBio	NSABB Full Board Meeting (October 22, 2014), In-person WG Meeting (July 23, 2015)
Rocco Casagrande, Ph.D.	Gryphon Scientific	NSABB Full Board Meetings (September 28, 2015 and January 7-8, 2016), In-person WG Meeting (November 9, 2015), National Academies Workshop (March 10-11, 2016)
R. Alta Charo, J.D.	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Susan Coller-Monarez, Ph.D.	U.S. Department of Homeland Security	In-person WG Meeting (July 23, 2015)
Louis (Tony) Cox, Ph.D., S.M.	Cox Associates	National Academies Workshop (March 10-11, 2016)
Mark Denison, M.D.	Vanderbilt University	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Dennis Dixon, Ph.D.	U.S. National Institutes of Health	NSABB Full Board Meeting (November 25, 2014)
Marianne Donker, Ph.D.	Ministry of Health, Welfare and Sport, Netherlands	In-person WG Meeting (July 23, 2015)
Philip Dormitzer, M.D., Ph.D.	Novartis Vaccines	National Academies Workshop (December 15, 2014)
Ruxandra Draghia-Akli, M.D., Ph.D.	European Commission	In-person WG Meeting (July 23, 2015), National Academies Workshop (March 10-11, 2016)
Rebecca Dresser, J.D.	Washington University in St. Louis	NSABB Full Board Meeting (September 28, 2015)
Paul Duprex, Ph.D.	Boston University, NEIDL Institute	NSABB Full Board Meeting (October 22, 2015)
Gerald Epstein, Ph.D.	White House Office of Science and Technology Policy	In-person WG Meeting (July 23, 2015)

Stephen Eubank, Ph.D.	Virginia Polytechnic Institute and State University	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016)
Scott Ferson, Ph.D.	Applied Biomathematics	NSABB Full Board Meeting (October 22, 2014)
David Fidler, J.D., M.Phil.	Indiana University, Bloomington	NSABB Full Board Meeting (January 7-8, 2016)
Harvey Fineberg M.D, Ph.D.	University of California, San Francisco	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Adam Finkel, Sc.D., M.P.P.	University of Pennsylvania Law School	National Academies Workshops (March 10-11, 2016)
Baruch Fischhoff, Ph.D.	Carnegie Mellon University	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Robert Fisher, Ph.D.	U.S. Food and Drug Administration	National Academies Workshop (March 10-11, 2016)
Ron Fouchier, Ph.D.	Erasmus Medical Center	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
David Franz, D.V.M., Ph.D.	Former Commander, United States Army Medical Research Institute for Infectious Diseases	In-person WG Meeting (July 23, 2015)
Christophe Fraser, Ph.D.	Imperial College	National Academies Workshop (December 15, 2014)
Richard Frothingham	Duke University	National Academies Workshop (March 10-11, 2016)
Keiji Fukuda, M.D., M.P.H.	World Health Organization	National Academies Workshop (March 10-11, 2016)
George F. Gao, D.V.M., D.Phil.	Chinese Academy of Sciences; Chinese Center for Disease Control and Prevention	National Academies Workshop (March 10-11, 2016)
Gigi Kwik Gronvall, Ph.D.	University of Pittsburgh Medical Center, Center for Health Security	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Charles Haas, Ph.D.	Drexel University	National Academies Workshop (December 15, 2014)
Andrew M. Hebbeler, Ph.D.	U.S. Department of State	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Ruthanne Huising, Ph.D., M.Sc.	McGill University	National Academies Workshop (March 10-11, 2016)
Gavin Huntley-Fenner, Ph.D.	Huntley-Fenner Advisors	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Jo Husbands, Ph.D.	Board on Life Sciences of the U.S. National Academies of Sciences, Engineering and Medicine	In-person WG Meeting (July 23, 2015), NSABB Full Board Meeting (January 7-8, 2016)
Michael Imperiale, Ph.D.	University of Michigan	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Thomas Inglesby, M.D.	University of Pittsburgh Medical Center, Center for Health Security	NSABB Full Board Meeting (October 22, 2014 and January 7-8, 2016)
Barbara Jasny, Ph.D.	Science	In-person WG Meeting (July 23, 2015), NSABB Full Board Meeting (January 7-8, 2016)
Daniel Jernigan, M.D., M.P.H.	U.S. Centers for Disease Control and Prevention	NSABB Full Board Meeting (January 7-8, 2016)
Barbara Johnson, Ph.D., R.B.P.	Biosafety Biosecurity International	National Academies Workshop (December 15, 2014)
John Kadvany, Ph.D.	Independent consultant on decision science	Full Board Meeting (January 7-8, 2016)
Joseph Kanabrocki, Ph.D., C.B.S.P.	University of Chicago	In-person WG Meeting (January 22, 2015), In-person WG Meeting (July 23, 2015)

Isidoros Karatzas, Ph.D.	European Commission	WG Meeting (February 16, 2016)
Yoshihiro Kawaoka, D.V.M., Ph.D.	University of Wisconsin, Madison	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), National Academies Workshop (December 15, 2014)
George Kemble, Ph.D.	3-V Biosciences	National Academies Workshop (December 15, 2014)
Lawrence Kerr, Ph.D.	U.S. Department of Health and Human Services	WG Meeting (November 5, 2015), National Academies Workshop (March 10-11, 2016)
Gregory Koblentz, Ph.D., M.P.P.	George Mason University	National Academies Workshop (December 15, 2014)
Todd Kuiken, Ph.D.	The Woodrow Wilson Center	In-person Meeting (July 23, 2015)
Robert Lamb, Ph.D., Sc.D.	Northwestern University; Howard Hughes Medical Institute	National Academies Workshop (December 15, 2014)
Linda Lambert, Ph.D.	U.S. National Institutes of Health	In-person WG Meeting (July 23, 2015)
Gabriel Leung, M.D., M.P.H.	University of Hong Kong	National Academies Workshop (March 10-11, 2016)
Carol Linden, Ph.D.	U.S. Biomedical Advanced Research and Development Authority	National Academies Workshop (December 15, 2014)
W. Ian Lipkin, M.D.	Columbia University	NSABB Full Board Meeting (October 22, 2014)
Marc Lipsitch, Ph.D.	Harvard School of Public Health	NSABB Full Board Meetings (October 22, 2014 and January 7-8, 2016), National Academies Workshop (December 15, 2014)
Patricia Long, J.D., LL.M.	U.S. Department of Health and Human Services	In-person WG Meeting (July 24, 2015)
Nicole Lurie, M.D., M.S.P.H.	U.S. Department of Health and Human Services	NSABB Full Board Meeting (October 22, 2014); In-person WG Meeting (July 23, 2015)
Eric Meslin, Ph.D.	Indiana University School of Medicine	NSABB Full Board Meeting (September 28, 2015)
Corey Meyer, Ph.D.	Gryphon Scientific	NSABB Full Board Meeting (September 28, 2015), In-person WG Meeting (November 9, 2015)
Jonathan Moreno, Ph.D.	University of Pennsylvania	NSABB Full Board Meeting (January 7-8, 2016), National Academies Workshop (March 10-11, 2016)
Kara Morgan, Ph.D., M.S.E.S.	Battelle	National Academies Workshop (March 10-11, 2016)
Rebecca Moritz, M.S., C.B.S.P., S.M.(NRCM)	University of Wisconsin–Madison	National Academies Workshop (December 15, 2014)
Kalyani Narasimhan, Ph.D.	Nature Publishing Group	In-person WG Meeting (July 23, 2015)
Kimberly Orr, Ph.D.	U.S. Department of Commerce	In-person WG Meeting (July 23, 2015)
Michael Osterholm, Ph.D., M.P.H.	University of Minnesota	NSABB Full Board Meeting (October 22, 2015)
Kenneth Oye, Ph.D.	Massachusetts Institute of Technology	In-person WG Meeting (July 23, 2015)
Christopher Park	U.S. Department of State	In-person WG Meeting (July 23, 2015)
Jean Patterson, Ph.D.	Texas Biomedical Research institute	In-person WG Meeting (January 22, 2015)
Daniel Perez, Ph.D.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)

Janet Peterson, C.B.S.P.	University of Maryland	NSABB Full Board Meeting (October 22, 2014)
Philip Potter, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (January 7-8, 2016), National Academies Workshop (March 10-11, 2016)
David Relman, M.D.	Stanford University	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
David B. Resnik, J.D., Ph.D.	U.S. National Institutes of Health	NSABB Full Board Meeting (October 22, 2014)
Colin Russell, Ph.D.	University of Cambridge	National Academies Workshop (December 15, 2014)
Monica Schoch-Spana, Ph.D.	University of Pittsburgh Medical Center, Center for Health Security	National Academies Workshops (December 15, 2014 and March 10-11, 2016)
Stacey Schultz-Cherry, Ph.D.	St. Jude Children's Research Hospital	NSABB Full Board Meeting (October 22, 2014), National Academies Workshop (December 15, 2014)
Michael Selgelid, Ph.D.	Monash University	NSABB Full Board Meetings (September 28, 2015 and January 7-8, 2016), National Academies Workshop (March 10-11, 2016)
Ethan Settembre, Ph.D.	Seqirus	National Academies Workshop (March 10-11, 2016)
Richard Sever, Ph.D.	Cold Spring Harbor Laboratories Press; bioRxiv	In-person WG Meeting (July 23, 2015)
Michael Shaw, Ph.D.	Centers for Disease Control and Prevention	In-person WG Meeting (July 23, 2015)
Bill Sheridan, M.B., B.S.	BioCryst Pharmaceuticals Inc.	NSABB Full Board Meeting (October 22, 2014)
Kanta Subbarao, M.B.B.S., M.P.H.	National Institutes of Health	National Academies Workshop (December 15, 2014), NSABB Full Board Meeting (January 7-8, 2016)
Jill Taylor, Ph.D.	Wadsworth Center, NYS Department of Public Health	NSABB Full Board Meeting (January 7-8, 2016)
Robert Temple, M.D.	Food and Drug Administration	In-person WG Meeting (July 23, 2015)
Volker ter Meulen, M.D., Ph.D.	European Academies Science Advisory Council	National Academies Workshop (March 10-11, 2016)
Eileen Thacker, D.V.M., Ph.D., D.A.C.V.M.	U.S. Department of Agriculture	In-person WG Meeting (July 23, 2015)
Silja Vöneky, Prof., Dr., jur.	University of Freiburg; German Ethics Council	National Academies Workshop (March 10-11, 2016)
Robert Webster, Ph.D.	St. Jude Children's Research Hospital	National Academies Workshop (December 15, 2014)
Jerry Weir, Ph.D.	U.S. Food and Drug Administration	National Academies Workshop (December 15, 2014)
Robbin Weyant, Ph.D., R.B.P. (ABSA)	U.S. Centers for Disease Control and Prevention	National Academies Workshop (December 15, 2014), In-person WG Meeting (July 23,2015)
Beth Willis	Co-founder, Frederick Citizens for Bio-lab Safety	NSABB Full Board Meeting (January 7-8, 2016)
Carrie Wolinetz, Ph.D.	U.S. National Institutes of Health	NSABB Full Board Meetings (May 5, 2015 and January 7-8, 2016)

Table 1B. Public Commenters. Individuals and organizations that provided written or oral public comments to the NSABB via email and/or at NSABB meetings.

Commenter	Affiliation/Location (if provided)
Regine Aalders, M.Sc.	Embassy of the Netherlands, Washington, D.C.
Richard S. Adams	
Ralph Baric, Ph.D.	University of North Carolina at Chapel Hill
RADM Kenneth W. Bernard, M.D.	U.S. Public Health Service (ret.)
Rocco Casagrande, Ph.D.	Gryphon Scientific
Rolan O. Clark	
Derrin Culp	White Plains, New York
Annie De Groot M.D.	EpiVax Inc.
Mark Denison, M.D.	Vanderbilt University
Nicholas Evans, Ph.D.	University of Pennsylvania
David S. Fedson, M.D.	Sergy Haut, France
Ron Fouchier, Ph.D.	Erasmus Medical Center
Gregory Frank, Ph.D.	Infectious Diseases Society of America
Matthew Frieman, Ph.D.	University of Maryland
Deborah Gold, M.P.H., C.I.H.	Pacifica, California
Peter Hale	Foundation for Vaccine Research
Elizabeth Hart	Adelaide, South Australia
Denise Hein	
Thomas Inglesby, M.D.	University of Pittsburgh
Tyler John	National Institutes of Health
Laura H. Kahn, M.D., M.P.H., M.P.P.	Woodrow Wilson School of Public and International Affairs, Princeton University
Andy Kilianski, Ph.D.	National Research Council Fellow at US Army
Lynn C. Klotz, Ph.D.	Center for Arms Control and Non-proliferation
Bill Kojola	Silver Spring, Maryland
F. Gerard Lelieveld	The Hague, Netherlands
Marc Lipsitch, Ph.D.	Harvard School of Public Health
Kim R. Loll	Frederick County & City Containment Laboratories Community Advisory Committee

Corey Meyer, Ph.D.	Gryphon Scientific
Carlos S. Moreno, Ph.D.	Emory University School of Medicine
Kara Morgan, Ph.D.	Battelle
Peter Murakami	Baltimore, Maryland
Daniel O'Connell	Albany, Oregon
Megan Palmer, Ph.D.	Center for International Security and Cooperation, Stanford University
Dustin Phillips	Louisville, Kentucky
Stanley Plotkin, M.D.	University of Pennsylvania
Ryan Ritterson	Gryphon Scientific
George Rudy	Frederick County & City Containment Laboratory Community Advisory Committee
Steven L. Salzberg, Ph.D.	Johns Hopkins University School of Medicine
Shannon Scott	
Billie Sellers	
Nariyoshi Shinomiya, M.D., Ph.D.	National Defense Medical College, Japan
Lone Simonsen, Ph.D.	George Washington University
Andrew Snyder-Beattie	Future of Humanity Institute, University of Oxford
Charles R. Stack, M.P.H.	University of Illinois at Chicago
Kanta Subbarao, M.B.B.S., M.P.H.	National Institutes of Health
John Steel, Ph.D.	Emory University
Kimball Ward	
Simon Warne, Ph.D.	UK Scientific Advisory Committee on Genetic Modification
Gary Whittaker, Ph.D.	Cornell University
Frances Williams, R.N., M.S.	Frederick Citizens for Bio-lab Safety
Beth Willis	Frederick Citizens for Bio-lab Safety
David Wolinsky	Fredrick, Maryland
American Association of Immunologists	American Association of Immunologists (AAI)
Infectious Diseases Society of America	Infectious Diseases Society of America (IDSA)

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http://ec.europa.eu/research/participants/data/ref/h2020/other/hi/guide_research-dual-use_en.pdf

European Commission Guidance note — Research with an exclusive focus on civil applications.
http://ec.europa.eu/research/participants/data/ref/h2020/other/hi/guide_research-civil-apps_en.pdf

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http://ec.europa.eu/research/participants/data/ref/h2020/other/hi/guide_research-misuse_en.pdf

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Appendix F. NSABB Framework for Guiding the Risk and Benefit Assessments

The National Science Advisory Board for Biosecurity developed the recommendations contained in the following section as part of its charge stemming from the *U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS viruses*. As part of its charge, the NSABB was to provide advice on the design, development, and conduct of risk and benefit assessments for gain-of-function studies. The *Framework for Guiding the Conduct of Risk and Benefit Assessments of Gain-of-Function Research* which follows fulfills this portion of the NSABB's charge and was developed with the aim of helping to generate risk and benefit assessments that would provide information that would allow the NSABB to make sound, evidence-based recommendations.

FRAMEWORK FOR CONDUCTING RISK AND BENEFIT ASSESSMENTS OF GAIN-OF-FUNCTION RESEARCH

RECOMMENDATIONS OF THE NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

National Science Advisory Board for Biosecurity
MAY 2015

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PREAMBLE

The National Science Advisory Board for Biosecurity developed the recommendations contained in this document as part of its charge stemming from the *U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS viruses*, issued on October 17, 2014. As part of its charge, the NSABB is to 1) provide advice on the design, development, and conduct of risk and benefit assessments for gain-of-function studies, and 2) provide formal recommendations on the conceptual approach to the evaluation of proposed gain-of-function studies. This document was unanimously approved by the committee on May 5, 2015 and fulfills the first portion of the NSABB's charge. The recommendations in this document will guide the National Institutes of Health as it commissions a formal assessment of the potential risks and benefits associated with gain-of-function research involving pathogens with pandemic potential. The results of the risk and benefit assessments will inform the NSABB as it develops its recommendations to the United States Government about how to evaluate such studies.

BACKGROUND AND INTRODUCTION

Most genetic manipulations of microorganisms do not raise significant safety or security concerns; these studies are routinely conducted for valid scientific purposes using non-pathogenic organisms or biologic systems and are subject to appropriate Federal and institutional oversight. However, safety and security concerns may arise when certain types of manipulations, which introduce stable genetic mutations, are employed to better understand some pathogens or toxins, sometimes enhancing the ability of those agents to harm their hosts.

Recently, the phrase "gain-of-function (GOF) research" has come to describe certain studies that increase the ability of a pathogen to cause disease. This phrase achieved prominence after two groups published findings demonstrating that highly pathogenic avian influenza H5N1 viruses with a small number of engineered mutations became transmissible between mammals by respiratory droplets.[77,78] Such studies were undertaken to help define the fundamental nature of human-pathogen interactions, with the goal of enabling assessment of the pandemic potential of emerging infectious agents, informing public health and preparedness efforts, and furthering medical countermeasure development. However, such GOF studies may entail biosafety and biosecurity risks, and significant concerns have been raised about whether these studies generate information that could be misused to cause harm or whether the modified viruses could pose a pandemic threat if they were to be accidentally or intentionally released.

In 2012, a voluntary suspension of certain GOF studies involving highly pathogenic avian influenza H5N1 viruses was undertaken by the influenza research community.[79] During that time, policymakers considered whether certain GOF studies should be conducted using Federal funds, and if so, how those studies could be safely conducted. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) issued new biosafety guidelines for working with highly pathogenic avian influenza strains.[80,81] The U.S. Department of Health and Human Services (HHS) developed a framework for guiding its funding decisions about projects that may generate highly pathogenic H5N1 viruses that are transmissible between mammals by respiratory droplets.[82] This funding framework was later expanded to include H7N9 influenza viruses as well.[83] Under this framework, HHS considers newly submitted research project proposals involving certain GOF studies for their scientific and public health merits as well as associated biosafety, biosecurity, and dual use risks. HHS also identifies appropriate risk mitigation measures that are required. Studies that are deemed acceptable for funding may then proceed in accordance with any agreed-upon risk mitigation measures.

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Given the biosafety incidents in U.S. Federal laboratories during the summer of 2014 and renewed concerns regarding laboratory safety and biosecurity, the U.S. government (USG) determined that the risks and benefits of GOF research must be re-evaluated.[84] A robust and broad deliberative process that will result in the adoption of a new Federal GOF research policy (which will apply to research funded by U.S. agencies whether conducted in the U.S. or abroad) has been undertaken. While this process takes place, the USG has instituted a pause in the provision of new USG funding for certain GOF research involving influenza, Middle East Respiratory Syndrome coronavirus (MERS) or Severe Acute Respiratory Syndrome coronavirus (SARS) viruses—pathogens determined to have pandemic potential. Restrictions on new funding apply as follows:

> *New USG funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. This restriction would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity.*

In parallel, the USG has encouraged the research community (both those who receive USG funding and those who do not) to join in adopting a voluntary pause on any on-going research that involves the types of studies that are subject to the funding restriction above.

The deliberative process involves both the National Science Advisory Board for Biosecurity (NSABB) and the National Academies, and involves explicit evaluation of the possible risks and potential benefits of GOF research with potential pandemic pathogens. The NSABB serves as the official Federal advisory body for providing advice on oversight of this area of dual use research. The NSABB is providing the USG with specific recommendations regarding a conceptual approach to the evaluation of proposed GOF studies. The National Research Council and the Institute of Medicine of the National Academies are convening forums to engage the life sciences community as well as to solicit feedback from scientists and the public on optimal approaches to ensure effective Federal oversight of GOF research. These forums involve discussion of principles important for the design of risk and benefit assessments of GOF research and of NSABB draft recommendations.

The final NSABB recommendations and the discussions at the National Academies forums will be taken into consideration by the USG during the development and adoption of a new USG policy governing the funding and conduct of GOF research.

Thorough and scientifically rigorous risk and benefit assessments of GOF research involving pathogens with pandemic potential are needed to inform the deliberative process, and to provide the NSABB and the USG with objective and comprehensive information about the risks and benefits associated with certain types of GOF research. The USG has determined that an independent contractor will conduct the risk and benefit assessments (RA and BA). The contractor will provide personnel and expertise for conducting the RA and BA on certain GOF research involving pathogens with pandemic potential. The RA and BA are to be comprehensive, sound, and credible and must be able to withstand rigorous scrutiny by a variety of stakeholders. The contractor's analyses are to be guided by the overall guiding

[84] *U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS viruses*, October 17, 2014.

principles described herein. In planning and conducting the RA and BA, the contractor will take into account issues raised by recent biosafety incidents in USG laboratories.

While the funding pause and the RA and BA are limited to specific pathogens,[85] products of the RA and BA are intended to inform broader NSABB deliberations, which will involve recommendations on a conceptual approach to the evaluation of proposed GOF studies that may extend to other high-consequence pathogens. NSABB recommendations will inform the USG as it develops and adopts policies about whether certain types of GOF studies on high-consequence pathogens with pandemic potential should be supported and, if so, how such funding proposals should be evaluated.

A private contractor will conduct the RA and BA; however, the process is intended to be a cooperative effort involving participation by NIH and the NSABB, and informed by discussions held at the National Academies forums. The NIH Office of Science Policy is managing the overall deliberative process, providing the interface and facilitating the communications between the contractor and other entities, and overseeing the work by the contractor. The studies and resulting reports must comply fully with USG requirements, both procedurally and analytically, using existing guidance from Federal agencies and peer-reviewed sources and well-established methods. Concerns of other stakeholders, in addition to the USG, must be considered.

THE CHARGE TO THE NSABB

The NSABB has been charged with providing advice on the design, development, and conduct of risk and benefit assessments, and with providing recommendations to the USG on a conceptual approach to the evaluation of proposed GOF studies. In developing its recommendations, the NSABB will consider: the results of the RA and BA; the spectrum of potential risks and benefits associated with GOF studies; alternative methods that may be employed to yield similar scientific insights or benefits, while reducing potential risks; public discussions hosted by the National Academies; and any additional consultations with relevant subject matter experts, as needed, to ensure that all appropriate expertise is brought to bear on the issues. In advising on the design and conduct of the RA and BA, the NSABB will recommend assumptions to be included in the risk assessment; evaluate the scope and methodologies to be used in the risk assessment; consider the adequacy of the scenarios in the risk assessment and propose additional scenarios to address other concerns or factors, as appropriate; advise on the assessment of the benefits, including types of benefits that should be examined and methods for examining them; and provide advice at key milestones in the conduct of the RA and BA.

To satisfy this charge, the NSABB will convene, deliberate, and provide two deliverables to the USG:

- **Deliverable 1.** Advice on the design, development, and conduct of risk and benefit assessments.
- **Deliverable 2.** Formal recommendations on the conceptual approach to the evaluation of proposed GOF studies.

The framework outlined herein, and subsequent input provided by the NSABB at key milestones throughout the conduct of the RA and BA, are intended to satisfy Deliverable 1.

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[85] *U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS, and SARS viruses*, October 17, 2014.

THE NSABB'S PROCESS

In order to accomplish its charge regarding Deliverable 1, the NSABB established a Working Group (WG), composed of 13 NSABB members with a broad range of expertise including microbiology, biodefense, ethics, biosecurity, national security, biosafety, public health, and other relevant areas. The WG also included non-voting *ex officio* members from Federal agencies who contributed expertise in virology, national security, ethics, foreign policy, and other areas. The group convened during the period of December 2014 through April 2015 by telephone conference calls and held a one-day in-person meeting to discuss the design and conduct of the RA and BA and to begin to identify the information necessary to inform the Board's final recommendations to be issued in Deliverable 2. The discussions ranged broadly and included general concepts of overall importance as well as specific details that the contractor should consider and include as the RA and BA proceed. The WG's findings were consolidated into a series of recommendations that were discussed and developed further, and ultimately approved by the full Board on May 5, 2015. The recommendations in this Framework are intended to guide the NIH as it works with the contractor performing the RA and BA such that the assessments will be conducted in a way that will provide information that allows the NSABB to make sound, evidence-based recommendations. The NSABB acknowledged the strengths and limitations associated with such assessments, which primarily involve scientific and technical input, and has noted that other information, such as consideration of ethical, legal, and other viewpoints, should inform its final recommendations (Deliverable 2).

In guiding the design of the RA and BA, the NSABB focused on issues specific to GOF studies but noted that some other directly relevant studies are important for comparison and should be included in the assessments. Although the RA and BA focus on specific experiments and scenarios, the scope is intended to be sufficient to allow evaluation of the risks and benefits of not just single experiments, but also whole research programs to inform decisions pertaining to the entire USG research portfolio related to GOF studies with high consequence pathogens with pandemic potential.

Finally, an issue of central importance to the entire deliberative process is public trust in the scientific enterprise. A possible negative outcome associated with the GOF issue is the loss of public trust if a laboratory accident involving modified strains were to occur or if GOF research were intentionally misused to cause harm. Loss of public trust is a serious concern and its impact could be felt widely across the scientific community. The deliberative process should be conducted with an eye toward maintaining public trust in the scientific enterprise and oversight of scientific research. To help ensure public trust, and to ensure the NSABB's deliberations are informed by broad input and diverse perspectives, the NSABB seeks to maximize stakeholder input and public engagement during the deliberative process. Of note, the deliberative process includes public forums hosted by the National Academies that are intended to gather input and foster broad discussions by the scientific and other stakeholder communities. The first forum was held in December 2014;[86] a second will be held later in the process. Additionally, NSABB meetings are open to the public and the Board encourages attendees to provide comments, either verbally or in writing. The NSABB encourages comments and input at any time, which can be submitted by emailing NSABB@od.nih.gov.

[86] *Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop*. National Research Council and the Institute of Medicine of the National Academies. The National Academies Press, Washington D.C., 2015. www.nap.edu.

RECOMMENDATIONS REGARDING THE DESIGN AND CONDUCT OF THE RA AND BA

Guiding Principles

Listed below (not necessarily in order of importance) are guiding principles that should underpin the risk and benefit assessments. These principles should inform and guide the contractor's efforts in performing the risk and benefit assessments.

1. There are potential risks and benefits associated with certain GOF life sciences research that should be formally and rigorously identified and analyzed. The possible risks and benefits of not doing this work also need to be thoroughly examined.

2. Alternative experimental approaches to GOF experiments that may provide the same or similar outcomes or additional/different benefits, without the same risks, should be identified and their relative risks, benefits, and limitations thoroughly and impartially analyzed. There may be different risks and benefits associated with these alternatives.

3. The RA and BA processes should start with a clear articulation of their purposes. The issues must be framed appropriately, with specific, relevant questions to be answered. The RA and BA should be conceptualized so as to provide information that is useful and informative for guiding NSABB recommendations about whether or not and how to pursue the types of scientific studies that are the subject of the assessments.

4. The scope of the RA and BA must be sufficiently comprehensive and delineated, with all aspects of the problem being clearly defined and considered at the outset. While the scope must be sufficiently detailed, it also must be appropriately narrowed to the particular subset of studies whose risks may be especially significant.

5. The concepts of clarity, transparency, consistency, and reasonableness must underpin the RA and BA. The processes must be well-documented and the final results and their interpretations should be clearly described and presented.

6. The assessments must be objective, scientifically rigorous, comprehensive, credible, and reasonable. Analyses of potential risks and benefits should be based on existing guidance, use real data to the extent possible, and employ established, tested, and peer-accepted methods. The RA and BA should include both qualitative and quantitative analyses to the extent feasible.

7. Analyses should examine the impact of risk mitigation strategies and practices, the effect of public health interventions, and whether countermeasures are effective against novel strains, as well as how these strategies are actually employed, which may involve human error, crisis conditions, or other factors that decrease their effectiveness.

8. The data used are critical to conducting the risk and benefit assessments. Sources of data, quality of data, assumptions made in analyses, limitations of data, and areas where more data are needed all require explicit documentation. However, insufficient or lack of quality data should not be grounds for not addressing issues pertinent to the goals of the assessments.

Particular consideration must be given to issues of uncertainty[87] and sensitivity[88] in presenting results. Ranges and bounds should be used to reflect the level of confidence in the results.

9. The RA should address what could go wrong as a result of conducting GOF research, and the probability and consequences of such events. The BA should address what beneficial outcomes might result from such research, how probable they are, the magnitude of their effects, and a realistic timeframe for realizing the benefits. Both risks and benefits may depend on other factors and have different timeframes. Any assumptions regarding factors that must be present for the risks or benefits to be realized should be explicitly identified.

10. The focus of the assessments should be on research studies conducted within the U.S. or supported by U.S. funding and conducted outside of the U.S., but should take into account the fact that laboratories throughout the world that are not funded by the U.S. government may also be conducting similar studies.

11. These principles largely apply to both the RA and BA; however, the benefits are not just reduction of the risks included in the risk assessment. It may not always be feasible to express risks and benefits in the same terms, but an effort should be made to do so when possible.

12. The RA must encompass a range of scenarios including "maximum reasonable foreseeable events" (i.e., worst case) as well as those with a range of probabilities. Low probability but high consequence events deserve particular attention. Both intentional (malevolent) and accidental events should be included in the analyses.

Pathogens and Pathogen Characteristics

Listed below are pathogens that are recommended for inclusion in the RA and BA to provide information about the risks and benefits associated with GOF research involving these specific agents; however, the NSABB's ultimate policy recommendations need not be limited to these specific pathogens. The risks and benefits analyzed in the assessments are intended to be representative of those associated with similar agents and experiments that may arise in the future. Most pandemics are associated with respiratory transmission, so agents in this category are of overarching concern. The NSABB considered adding a variety of agents, viral and bacterial, as well as agents having different transmission routes that might gain the property of respiratory transmission. The NSABB also discussed the pathogen characteristics that are most concerning.

[87] Uncertainty is the lack or incompleteness of information. Quantitative uncertainty analysis attempts to analyze and describe the degree to which a calculated value may differ from the true value; it sometimes uses probability distributions. Uncertainty depends on the quality, quantity, and relevance of data and on the reliability and relevance of models and assumptions used to fill data gaps. From *Science and Decisions: Advancing Risk Assessment*. National Research Council of the National Academies, The National Academies Press; Washington DC. 2009.

[88] Sensitivity is the degree to which the outputs of a quantitative assessment are affected by changes in selected input parameters or assumptions. From *Science and Decisions: Advancing Risk Assessment*. National Research Council of the National Academies, The National Academies Press; Washington DC. 2009.

Pathogens recommended for inclusion in the RA and BA:

1. **Influenza viruses.** Because of the significant differences among influenza strains, the NSABB recommends that three distinct strains be analyzed. These are:

    a. Seasonal influenza (e.g., currently circulating or historical H1N1, H3N2, and influenza B strains for which a significant portion of the general population has pre-existing immunity)
    b. Highly pathogenic avian influenza virus H5N1
    c. Low pathogenic avian influenza virus H7N9

2. **SARS-CoV**

3. **MERS-CoV**

Pathogen characteristics recommended for consideration in the RA and BA:

The RA and BA should include analysis of the risks and benefits associated with GOF experiments that are anticipated to increase the pandemic potential of pathogens. Toward this end, the following characteristics, which may be conferred to pathogens during the conduct of GOF studies, should be considered:

1. Enhanced pathogen production as a result of changes in the replication cycle or growth.

2. Enhanced morbidity and mortality in appropriate animal models.

3. Enhanced transmission in mammals (e.g., increased host or tissue range, altered route of transmission, infectivity above a certain threshold determined in an appropriate animal model).

4. Evasion of existing natural or induced immunity.

5. Resistance to drugs or evasion of other medical countermeasures such as vaccines, therapeutics, diagnostics.

Risk Categories

In order for the contractor to plan and conduct the risk assessment so that it will ultimately meet the needs of the NSABB, the scope of possible risks must be defined at the outset. The risk assessment should particularly examine any risks that are unique to GOF studies and examine the relative risks of GOF research compared to alternative approaches. It is important that all reasonable categories of risks be examined. Listed below are the categories of risks that the NSABB recommends be considered in the RA. There is some overlap between the categories, and of note, potential national biosecurity risks that should be considered are associated with most of the categories. For each of the risk categories, both intentional and accidental events that lead to risk should be considered, as appropriate. In addition, the analysis should consider the risks associated with certain GOF studies in the context of currently existing risks associated with the broader, national biomedical research portfolio and from the perspective of past experience. The RA should also consider the additive risks associated with conducting relevant GOF

studies at multiple locations. Where there are case studies or known examples of events that document various risks, these should be compiled and selected examples incorporated into the RA report.

1. **Biosafety:** Biosafety risks are those generally associated with laboratory accidents. Assessing these risks should include the magnitude of exposures, initial infections, transmission leading to secondary infections, and outbreaks in humans or animals. The issue of novel pathogenic strains for which we may be unprepared needs particular attention. The association of laboratory personnel with intermediary hosts should also be considered. The RA should evaluate the effect that public health interventions and occupational health and staff monitoring programs have on the risks posed by novel pathogens resulting from GOF studies, as compared to existing pathogens. The assessment should consider how the capabilities and containment features of the lab doing the work influence risk. The risks to lab workers and to the general public should be analyzed separately.

2. **Physical and personnel security (biosecurity):** Biosecurity risks are those associated with crime and terrorism involving pathogens resulting from GOF studies and would take into account the physical security of pathogens, risks associated with shipping and transporting pathogens, and the risk of illegitimate acts by "insiders," or laboratory employees. Biosecurity risks include physical breach, theft, loss or intentional release by lab personnel, malevolent acts, and terrorism. The RA should include consideration of the types of actors who would seek to misuse life sciences research information and materials as well as their capabilities to do so. The analysis should also consider specifically how the studies in question could be misused, whether terrorists might target labs to gain access to materials or scientific expertise, and include estimates of how great the threats may be.

3. **Proliferation:** The RA should consider how pursuing certain GOF studies may lead to expanded amounts of that research and, as a result, increased risk (biosafety, biosecurity, and others). Proliferation might occur if certain studies become standard or typical, or, conversely, if unpublished studies (due to safety or security concerns) are repeated, unwittingly by others. This analysis should take into account that biosafety standards vary in different countries and settings.

4. **Information risk:** Information risks are those associated with how the information generated by GOF studies, if made publically available, could enable others throughout the world to replicate such studies or generate pathogens for malevolent actions or threats to national security. Intellectual property threats may also be considered here.

5. **Agricultural:** This involves the risks to agriculturally-relevant animals such as pigs or chickens if a laboratory-modified pathogen produced during GOF studies was to be intentionally or accidentally released into populations of these animals. This also includes risks resulting from interaction between humans and other reservoir hosts.

6. **Economic risks:** Economic risks include monetary costs associated with releases of pathogens resulting from GOF studies, including loss of productivity, agricultural damage, liability, and the issue of accountability. Opportunity costs might also be considered.

7. **Loss of public confidence:** It is important to consider the possible loss of public trust in the scientific enterprise that might result if a laboratory accident involving modified pathogens were

to occur or if products or information from GOF research were intentionally misused to cause harm. Loss of public trust is a serious concern and its impact could be felt widely across the scientific community.

Benefit Categories

In order for the contractor to plan and conduct the BA so that it will ultimately meet the needs of the NSABB, the scope of potential benefits that may result from GOF research must be defined at the outset. The BA should particularly examine any unique benefits that could be realized as a result of GOF studies and examine the relative benefits of GOF research compared to alternative approaches. It is important that all reasonable categories of benefits be examined. Listed below are several categories of benefits that the NSABB recommends for inclusion in the BA. It should be noted that national security dimensions to the benefits associated with several categories should be considered. The NSABB notes that some benefits may only accrue if other associated events also take place. The NSABB also acknowledges the difficulty of analyzing some benefits, particularly those with long-term timeframes.

1. **Scientific knowledge**: These benefits include analysis of the types of scientific information that could be generated from GOF research, and an assessment of the value of such information for understanding the agents/diseases being studied (or other agents/diseases). The assessment should consider ways to quantify these benefits if possible. The BA should also analyze whether GOF research generates (or is likely to generate) unique scientific information that expands the knowledge base in ways that other research approaches cannot.

2. **Biosurveillance:** These benefits would include those relevant to the processes of gathering, integrating, analyzing, interpreting, and communicating essential information that might relate to disease activity and threats to human, animal, or plant health.[89] Specifically, the potential benefits of relevant GOF studies should be examined for benefits to:

   a. **Public Health Surveillance[90]:** How GOF research may contribute to efforts to improve public health by aiding detection and monitoring of pathogens in the real world, or help to better recognize or predict outbreaks in human populations, and inform decision-making.

   b. **Agricultural and domestic animal surveillance:** How GOF research may contribute to efforts to improve agricultural health by aiding detection and monitoring of pathogens in

[89] The National Association of County and City Health Officials, http://naccho.org/topics/emergency/biosurveillance/index.cfm, defines biosurveillance as a process of gathering, integrating, interpreting, and communicating essential information that might relate to disease activity and threats to human, animal, or plant health. For the public health professional, biosurveillance activities range from standard epidemiological practices to advanced technological systems, utilizing complex algorithms.
[90] The World Health Organization, http://www.who.int/topics/public_health_surveillance/en/, defines public health surveillance as the continuous, systematic collection, analysis and interpretation of health-related data needed for the planning, implementation, and evaluation of public health practice. Such surveillance can serve as an early warning system for impending public health emergencies; document the impact of an intervention, or track progress towards specified goals; and monitor and clarify the epidemiology of health problems, to allow priorities to be set and to inform public health policy and strategies. CDC defines public health surveillance as the ongoing, systematic collection, analysis, and interpretation of health data, essential to the planning, implementation and evaluation of public health practice, closely integrated with the dissemination of these data to those who need to know and linked to prevention and control. See http://www.cdc.gov/niosh/topics/flu/surveillance.html.

food-producing, domestic, or other animals so as to help to better recognize or predict outbreaks in such animals, and inform decision-making.

c. **Wildlife surveillance:** How GOF research may contribute to the improvement of surveillance in wildlife by aiding detection and monitoring of pathogens, or help to better recognize or predict outbreaks in such animals, and inform decision-making.

3. **Medical countermeasures:** For the following three benefits in particular, the benefit assessment should examine the relative benefits of GOF research compared to alternative approaches. The assessment should also consider whether, and if so, how, GOF research yields unique information that may not otherwise be possible.

   a. **Therapeutics**: How the research is likely to aid discovery and development of new or more effective therapeutics.

   b. **Vaccines:** How the research is likely to aid development and selection of new or more effective vaccines.

   c. **Diagnostics:** How the research is likely to aid development of new or better diagnostic methods and products.

4. **Informing policy decisions:** How information gained from GOF studies contributes, or is likely to contribute, to public health preparedness decisions such as informing countermeasure stockpiling decisions, guiding decisions about strain selection for vaccine development, or informing decisions about whether and how to mobilize resources or issue guidance in response to a newly emergent pathogen.

5. **Economic benefits:** Possible gains (monetary, employment, labor productivity, etc.) and cost savings associated with the results/outcomes of GOF studies, such as diminished health care costs due to vaccines or therapeutics, or other positive impacts on the economy.

Historical Perspectives from Analysis of Past Experiences

Naturally-occurring epidemics and pandemics can provide helpful background information that might inform the discussion about the risks associated with the infectious agents that are subjects of RA and BA. There is significant historical data on the mortality and morbidity associated with seasonal and pandemic influenza, as well as more recent data on the other pathogens recommended for inclusion the RA and BA studies. However, there are complexities and limitations to interpreting these data and trends that require further analysis. Valuable historical perspectives about past outbreaks of seasonal and pandemic influenza, SARS, and MERS viruses could be obtained by conducting quantitative analyses of global pathogen-associated morbidity and mortality. This information will supplement the RA and BA being undertaken as part of the deliberative process on GOF research, and will help inform the development of the NSABB's final recommendations (Deliverable 2).

Specifically, the NSABB recommends that an analysis be done for each pathogen, which summarizes existing data and information and, to the extent possible, includes:

1. Global morbidity and mortality data associated with seasonal influenza, pandemic influenza, SARS, and MERS, and trends in these data over time.

2. If applicable, comparison of the morbidity and mortality associated with seasonal and pandemic illness.

3. Historical information about the impact of illness on food production, particularly the swine and poultry industries.

4. Description of how the data utilized were collected, interpreted, and analyzed.

5. Qualitative review of the impact of vaccines and therapeutics on pathogen-associated morbidity and mortality.

Scenarios and Events to be Included in the RA

The RA should be based on a series of events that might occur during the course of conducting GOF research. It is anticipated that the contractor will develop a large list of possible events and scenarios that might be included. Because of time and resource constraints, only a subset will be analyzed in depth; however, it is important to define the total range of reasonably likely events so that the ones that are analyzed will be representative of the risks anticipated to be associated with GOF research more broadly. Scenarios should include analysis of the effects of risk mitigation approaches and include realistic examples where mitigation is effective and where it fails in some way. The analyses should incorporate examples that account for variability between labs and their practices.

Development and Selection of Events and Scenarios

Listed below are recommendations, derived from the Guiding Principles identified above, which should guide the contractor as specific scenarios are developed and proposed for analysis.

1. Scenarios and events should be scientifically, politically, and socially accurate and credible.

2. To the extent possible, events and scenarios should be realistic and based on actual examples, possibly including the recent laboratory accidents at Federal facilities.

3. The overall range of scenarios should encompass high and low risk events, high and low probability events, and maximum reasonably foreseeable (highly unlikely, but still credible) events.

4. The scenarios should involve events that are of concern to stakeholders, including the public, and include types that involve experimental manipulations that ultimately may be determined to be prohibited under any circumstances.

5. Scenarios involving security threats should be plausible but not necessarily based on specific, real-life examples, given that the security landscape is constantly evolving. Such scenarios should involve consideration of the prior actions or expressed intent of hostile groups, current and reasonably achievable technical capabilities of these groups, and how readily security threats could be achieved or enabled by a certain type of GOF study.

Categories of Events and Scenarios

Listed below are types of events and scenarios that the NSABB recommends for consideration in the RA. The contractor should propose more specific scenarios based on these categories.

1. Accidents due to equipment failure, human error, and system malfunction
2. Events that lead to direct infection of lab worker(s)
3. Accidental direct release into the environment, with possible exposure of the public
4. Scenarios that lead to secondary transmission of disease in the community, starting with an infected lab worker
5. Incidents that result from security failures, either building systems or personnel
6. Incidents stemming from inventory errors and those involved with laboratory transitions, such as laboratories relocating, principal investigators retiring, students graduating, etc.
7. Scenarios involving the escape of an infected animal
8. Scenarios that result in health and/or economic impacts on important animal species, particularly those important to the food supply
9. Insider threats: an internal breach of security (e.g., disgruntled lab worker, infiltration of a lab by an individual with nefarious intent)
10. External threats: an external breach of security (e.g., crime, targeting of a lab for theft of agents or materials)
11. Production of novel pathogens for malevolent acts or other illegitimate purposes based on information published about the results of GOF research
12. Natural disasters (e.g., earthquake, hurricane, tornado)
13. Accidents resulting from conduct of GOF research under sub-standard biosafety/biocontainment conditions or practices, either in the U.S. or internationally
14. Scenarios based on alternative experimental approaches to GOF research

Types of Experiments in RA

The scope of research that is of concern must be clearly defined at the outset. Not all research that involves genetic manipulations to alter a pathogen's phenotype should be examined in the RA and BA. Listed below are types of experiments recommended for consideration in the RA and BA, but the NSABB's ultimate policy recommendations need not be limited to the specific experiment types included in the assessments. The following list includes experiment types that should be incorporated into

scenarios to be modeled in the RA. Importantly, inclusion of these types of experiments is not intended to condemn or condone them. The goal is to get a broad sense of the risks and benefits associated with different experimental manipulations in the context of the pathogens identified above, recognizing that not all permutations of risks, agents, and scenarios can practically be analyzed in depth.

1. Passage in animals with the intent to alter host range and generate mammalian adapted strains or to develop an animal model of disease
2. Genetic modifications and/or selection for traits that may increase pathogenicity or transmissibility
3. Manipulations resulting in better growth or enhanced replication, for example, to make a vaccine strain
4. Selection for drug-resistant mutants
5. Antigenic escape studies, i.e., selecting for pathogens that are not neutralized by certain antibodies, such as those generated in response to a vaccine or monoclonal antibodies
6. Alternative experiments to GOF that may yield similar scientific information

Biosafety Assumptions for the RA

In order to assess the risks associated with GOF experiments it is necessary to define the biosafety level (BSL) and other related conditions under which the work may take place because differences in working conditions may significantly affect the risk of an experiment and possible adverse results. In the United States, the *Biosafety in Microbiological and Biomedical Laboratories* and the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*)[91] provide biosafety guidance regarding the conduct of risk assessments, and determination of appropriate laboratory practices and physical containment for research conducted with specific agents. These guidelines apply to certain Federally-funded research conducted in the U.S. and abroad and are frequently used by non-Federally-funded institutions and other countries as the model for biosafety guidance. The NSABB recommends that the contractor carefully examine current guidance for biocontainment, biosafety practices, training, and occupational health plans and incorporate these features into their analysis.

Different countries have varying biosafety standards, and not all individuals replicating GOF work (especially including those intending to misuse their materials or results) will necessarily abide by biosafety standards. Therefore, to examine the range of risks associated with conducting GOF studies under different biosafety conditions, the NSABB recommends that risks associated with GOF studies involving each pathogen be assessed both 1) under biosafety conditions that are recommended under current guidance for the relevant studies and 2) under a range of biosafety conditions, so that the effects of different levels of mitigation, or lack thereof, can be determined. Also, the NSABB recommends that the effects of adequate or inadequate occupational medicine/medical surveillance programs, training, standard operating procedures, and administrative controls be examined. This approach will provide information the NSABB needs to make recommendations about the conditions under which certain GOF studies might be performed to maximize safety and minimize unnecessary

[91] http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

burden on the research. Finally, the NSABB recommends that the contractor investigate the status of biosafety guidance and biocontainment capabilities in other parts of the world, including guidance issued by the World Health Organization, and provide a summary of the findings.

Approaches and Methods for Assessing Risks and Benefits Associated with GOF Studies

The NSABB recommends that the following approaches be explored and employed by the contractor, as appropriate and reasonable, to assess the risks and benefits associated with relevant GOF studies. The contractor should examine these and other possible methods and identify those that might best be used to assess the specific categories of risks and benefits recommended above. Efforts to identify risks and benefits that are unique to GOF research should be emphasized.

1. Literature reviews and examination of knowledge indicators (e.g., science citation index), including consideration of quality and impact of information on the field.

2. Examination of commercialization indicators (e.g., number of patents), including considerations for quality and utility.

3. Interviews and consultations with a broad range of relevant experts about risks and benefits associated with GOF studies are highly recommended. Relevant experts might include those in various scientific disciplines, public health, clinical medicine, agriculture, private sector, global health, public policy, and national security, and should include experts both within and outside the United States. Consultations should include discussion of the important scientific questions remaining specifically for the pathogens being analyzed in the RA and BA and whether and how information from GOF studies may be utilized by relevant sectors. Discussions of how GOF studies contribute to research involving other pathogens with pandemic potential may also be useful. Interviews should also incorporate discussion of the perceived risks and benefits of alternatives to GOF studies.

4. Development of illustrative case studies or descriptions of instances where a GOF study has resulted in a specific risk or benefit.

5. Quantitative approaches to modeling the risks and benefits, particularly to public health. For instance, morbidity and mortality may be modeled for various scenarios of laboratory accidents, security breaches or intentional misuse, and/or public health responses. Additionally, if a GOF study were to accelerate vaccine or therapeutic production, it may be possible to model the positive effects on public health.

6. Quantitative approaches to modeling economic benefits and risks. For instance, if a GOF study would accelerate the development of a therapeutic or vaccine, the potential positive effects on jobs or productivity, as well as reduced health care costs in the event of a pandemic, might be estimated. In addition, the costs associated with an accidental release or malevolent act should be modeled.

7. Development of “event trees” illustrating processes leading to tangible events from GOF studies, employing expert elicitation to bound key events/nodes in processes.

Appendix G. NSABB Charter

THE SECRETARY OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20201

CHARTER

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

AUTHORITY

Authorized by 42 U.S.C. 217a, section 222 of the Public Health Service Act, as amended and Pub. L. 109–417, section 205 of the Pandemic and All-Hazards and Preparedness Act. The National Science Advisory Board for Biosecurity (NSABB) is governed by the provisions of the Federal Advisory Committee Act, as amended (5 U.S.C. app.), which sets forth standards for the formation and use of advisory committees.

OBJECTIVES AND SCOPE OF ACTIVITIES

The purpose of the NSABB is to provide, as requested, advice, guidance, and leadership regarding biosecurity oversight of dual use research, defined as biological research with legitimate scientific purpose that may be misused to pose a biologic threat to public health and/or national security. The NSABB will provide advice on and recommend specific strategies for the efficient and effective oversight of federally conducted or supported dual use biological research, taking into consideration both national security concerns and the needs of the research community to foster continued rapid progress in public health and agricultural research. Toward this end, the NSABB will also include providing strategies to raise awareness of dual use issues relevant to the life science and related interdisciplinary research communities.

DESCRIPTION OF DUTIES

The NSABB will be composed of subject matter experts who are not full-time employees of the Federal Government as well as ex officio members from Federal entities listed in the "Membership and Designation" section below, and will perform the following activities:

- Provide recommendations on the development of programs for outreach, education and training in dual use research issues for scientists, laboratory workers, students, and trainees in relevant disciplines.
- Advise on policies governing publication, public communication, and dissemination of dual use research methodologies and results.
- Recommend strategies for fostering international engagement on dual use biological research issues.

- Advise on the development, utilization and promotion of codes of conduct to interdisciplinary life scientists, and relevant professional groups.
- Advise on policies regarding the conduct, communication, and oversight of dual use research and research results, as requested.
- Advise on the Federal Select Agent Program, as requested.
- Address any other issues as directed by the Secretary of HHS.

AGENCY OR OFFICIAL TO WHOM THE COMMITTEE REPORTS

The NSABB will advise the Secretary of the Department of Health and Human Services (HHS), the Director of the National Institutes of Health (NIH), and the heads of all Federal entities that conduct, support or have an interest in life sciences research.

SUPPORT

Management and support services for the NSABB will be provided by the Office of Science Policy (OSP), within the Office of the Director, NIH. HHS and NIH staff will hold security clearances at the level of Secret or higher, as needed, to provide support to the NSABB.

ESTIMATED ANNUAL OPERATING COSTS AND STAFF YEARS

The estimated annual cost for operating the Committee, including compensation and travel expenses for members, but excluding staff support, is $274,900. The estimated annual person-years of staff support required is 1.5 at an estimated cost of $156,637.

DESIGNATED FEDERAL OFFICER

The Director, NIH, will assign a full-time or permanent part-time NIH employee to serve as the Designated Federal Officer (DFO) of the NSABB. In the event that the DFO cannot fulfill the assigned duties of the NSABB, one or more full-time or permanent part-time NIH employees will be assigned these duties on a temporary basis.

The DFO will approve or call all of the NSABB and subcommittee meetings, prepare and approve all meeting agendas, attend all Committee and subcommittee meetings, adjourn any meetings when it is determined to be in the public interest, and chair meetings when directed to do so by the Director, NIH, or the Director, OSP.

ESTIMATED NUMBER AND FREQUENCY OF MEETINGS

Meetings of the full committee will be held approximately two times within a fiscal year, and may be convened on an as-needed basis, at the call of the NSABB Executive Director or DFO. Meetings of the NSABB will be open to the public except as determined otherwise by the Secretary of Health and Human Services (Secretary), in accordance with subsection (c) of section 552b of Title 5 U.S.C. Notice of all meetings will be given to the public. In the event a portion of a meeting is closed to the public, as determined by the Secretary, in accordance with the Government in the Sunshine Act (5 U.S.C. 522b(c)) and the Federal Advisory Committee Act, a report will be prepared which will contain, as a minimum, a list of members and their business addresses, the Committee's functions, dates and places of meetings,

and a summary of the Committee's activities and recommendations made during the fiscal year. A copy of the report will be provided to the Department Committee Management Officer.

DURATION

Continuing.

TERMINATION

Unless renewed by appropriate action, the NSABB will terminate two years from the date this charter is filed.

MEMBERSHIP AND DESIGNATION

The NSABB will consist of not more than 25 voting members, including the Chair. Members will be appointed by the Secretary, HHS, in consultation with the heads of Federal departments and agencies that conduct or support life science research. The Secretary, HHS, will designate the Chair. All members will hold security clearances at the level of Secret or higher. Voting members are Special Government Employees and as such serve in their individual capacity as subject matter experts. None of these members serve as Representatives.

Areas of expertise to be represented on the NSABB, may include but are not be limited to:
Molecular Biology/Genomics
Microbiology (Bacteriology)
Microbiology (Virology)
Clinical Infectious Diseases/Diagnostics
Laboratory Biosafety and Biosecurity
Public Health/Epidemiology
Health Physicist/Radiation Safety
Pharmaceutical Production
Veterinary Medicine
Plant Health
Food Production
Bioethics
National Security
Military Biodefense Programs and Military Medicine
Intelligence
Biodefense
Law
Law Enforcement
Academia
Scientific Publishing
Industry Perspective
NIH Recombinant DNA Advisory Committee Experience/Perspective
Public Perspective
IBC perspective
Export Controls

There may be non-voting ex officio members from each of the following Federal entities:

- Executive Office of the President
- Department of Health and Human Services
- Department of Energy
- Department of Homeland Security
- Department of Veterans Affairs
- Department of Defense
- Department of the Interior
- Environmental Protection Agency
- Department of Agriculture
- National Science Foundation
- Department of Justice
- Department of State
- Department of Commerce
- Intelligence Community
- National Aeronautics and Space Administration
- Others as appropriate

Voting members will be invited to serve for overlapping terms of up to four years; terms of more than two years are contingent upon the renewal of the NSABB's Charter by appropriate action prior to its expiration. A voting member's term may be extended until a successor has been appointed.

A quorum for the NSABB and each of its subcommittees will consist of a majority of the appointed members eligible to vote. The nonvoting agency representatives will not be counted in calculating a quorum. Of the voting members, any who are recused from participating in an action on a particular issue, (e.g., due to a conflict of interest), will not be counted in calculating the quorum. All votes relating to any review of a recommendation by the NSABB will be open to the public unless the meeting has been closed to the public in accordance with the Government in the Sunshine Act and the Federal Advisory Committee Act.

SUBCOMMITTEES

As necessary, subcommittees and ad hoc working groups may be established by the NSABB Executive Director or DFO to perform functions within the Committee's jurisdiction. The advice/recommendations of the subcommittee/working group must be deliberated by the parent advisory committee. A subcommittee may not report directly to a Federal official unless there is statutory authority to do so.

Subcommittee membership may be drawn in whole or in part from the parent advisory committee. All subcommittee members may vote on subcommittee actions and all subcommittee members count towards the quorum for a subcommittee meeting. Ad hoc consultants do not count towards the quorum and may not vote. The Department Committee Management Officer will be notified upon establishment of each standing subcommittee and will be provided information on its name, membership, function, and estimated frequency of meetings.

RECORDKEEPING

Meetings of the Committee and its subcommittees will be conducted according to the Federal Advisory Committee Act, other applicable laws and Department policies. Committee and subcommittee records will be handled in accordance with General Records Schedule 6.2, Federal Advisory Committee Records, or other approved agency records disposition schedule. These records will be available for public inspection and copying, subject to the Freedom of Information Act, 5 U.S.C. 552.

FILING DATE

April 7, 2016

APPROVED

MAR 1 5 2016
Date

Sylvia M. Burwell
Sylvia M. Burwell

Appendix H. NSABB Roster

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[†] NASBB Working Group on the Design and Conduct of Risk and Benefit Assessments of Gain-of-Function Studies
[‡] NSABB Working Group on Evaluating the Risks and Benefits of Gain-of-Function Studies
[*] Retired NSABB Voting Member

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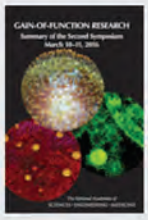

Gain-of-Function Research: Summary of the Second Symposium, March 10-11, 2016

DETAILS

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GAIN-OF-FUNCTION RESEARCH

Summary of the Second Symposium March 10–11, 2016

Piers Millett, Jo Husbands, Frances Sharples, and Audrey Thevenon,
Rapporteurs

Board on Life Sciences
Division on Earth and Life Studies

Board on Health Sciences Policy
Health and Medicine Division

Committee on Science, Technology, and Law
Policy and Global Affairs Division

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The **National Academy of Sciences** was established in 1863 by an Act of Congress, signed by President Lincoln, as a private, nongovernmental institution to advise the nation on issues related to science and technology. Members are elected by their peers for outstanding contributions to research. Dr. Ralph J. Cicerone is president.

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PLANNING COMMITTEE ON GAIN-OF-FUNCTION RESEARCH: THE SECOND SYMPOSIUM

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Acknowledgments

This workshop summary has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published workshop summary as sound as possible and to ensure that the summary meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this summary:

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Harvey Fineberg, Gordon and Betty Moore Foundation
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Peter Palese, Icahn School of Medicine at Mount Sinai
David Relman, Stanford University
Robert Webster, St. Jude Children's Research Hospital

Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the workshop summary before public release. The review of this summary

was overseen by Ellen Wright Clayton, Vanderbilt University, and Michael J. Imperiale, University of Michigan. They were responsible for making certain that an independent examination of this summary was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this summary rests entirely with the rapporteurs and the institution.

Contents

Acronyms and Abbreviations

APLU	Association of Public and Land-grant Universities
ASPR	Office of the Assistant Secretary for Preparedness and Response
BMBL	*Biosafety in Microbiological and Biomedical Laboratories* (CDC and NIH manual)
BWC	Biological Weapons Convention
DURC	dual use research of concern
EASAC	European Academies Science Advisory Council
EHS	environmental, health, and safety
EU	European Union
FACA	Federal Advisory Committee Act
FDA	Food and Drug Administration
GAO	Government Accountability Office
GEC	German Ethics Council
GOF	gain of function/gain-of-function
GOFRC	GOF research of concern
HHS	Department of Health and Human Services

IBC	Institutional Biosafety Committee
IDA	Institute for Defense Analyses
IRB	Institutional Review Board
IRE	Institutional Review Entity
MENA	Middle East and North Africa
MERS-CoV	Middle East respiratory syndrome coronavirus
NAS	National Academy of Sciences
NBACC	National Biodefense Analysis and Countermeasures Center
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NRC	National Research Council
NSABB	National Science Advisory Board for Biosecurity
NSABB WG	National Science Advisory Board for Biosecurity Working Group
OCS	Office of the Chief Scientist, Food and Drug Administration
OSP	Office of Science Policy, National Institutes of Health
OSTP	Office of Science and Technology Policy
PAPR	powered air purifying respirator
PI	principal investigator
R-BAT	Risk–Benefit Assessment Team
RAC	Recombinant DNA Advisory Committee
RBA	risk–benefit assessment
SARS-CoV	severe acute respiratory syndrome coronavirus
UPMC	University of Pittsburgh Medical Center
WHO	World Health Organization

1

Introduction

On March 10-11, 2016, the National Academies of Sciences, Engineering, and Medicine held a public symposium on potential U.S. government policies for the oversight of gain-of-function (GOF) research.[1] This was the Academies' second meeting held at the request of the U.S. government to provide a mechanism to engage the life sciences community and the broader public and solicit feedback on optimal approaches to ensure effective federal oversight of GOF research as part of a broader U.S. government deliberative process. Approximately 125 people attended the event in person, while more than 200 others watched the webcast.[2]

The first symposium, held in December 2014, examined the underlying scientific and technical questions surrounding the potential risks and

[1] As described in the Draft Working Paper of the National Science Advisory Board for Biosecurity, "the phrase 'gain-of-function research' has become synonymous with certain studies that enhance the ability of pathogens to cause disease. However, gain-of-function studies, as well as loss-of-function studies, are common in molecular and microbiology and form the foundation of microbial genetics. Changes to the genome of an organism, whether naturally occurring or directed through experimental manipulations in the laboratory, can manifest as altered phenotypes as biological functions are lost or gained. Such loss- and gain-of-function experiments allow investigators to understand the complex nature of host-pathogen interactions that underlie transmission, infection, and pathogenesis and can help attribute biological function to genes and proteins. The term 'gain-of-function' is generally used to refer to changes resulting in the enhancement or acquisition of new biological functions or phenotypes" (NSABB, 2015a: 7).

[2] The archived webcast, the presentation slides, and a complete transcript of the symposium are available on the project website at http://dels.nas.edu/Upcoming-Event/Gain-Function-Research-Second/AUTO-9-61-70-Q?bname=bls.

benefits of GOF research involving pathogens with pandemic potential (NRC, 2015).[3]

The second symposium focused on discussion of the draft recommendations regarding GOF research of the National Science Advisory Board for Biosecurity's (NSABB's) Working Group (WG). The recommendations are contained in a draft paper, which was released in December 2015 and discussed at an NSABB meeting on January 7-8, 2016 (NSABB, 2015a).[4] It also included discussion of a formal risk and benefit assessment (RBA) commissioned to inform the NSABB's work (Gryphon Scientific, 2015) and sessions devoted to current U.S. policy and the international developments that provide essential context for U.S. decisions. The public symposium did not attempt to develop consensus recommendations, but rather sought individual perspectives and robust discussion to inform the development of the NSABB's final recommendations. The Statement of Task for the symposium may be found in Box 1-1.

This report has been prepared by the rapporteurs as a factual summary of what occurred during the symposium. The planning committee's role was limited to organizing and convening the workshop. The views contained in the report are those of individual workshop participants and do not necessarily represent the views of all workshop participants, the planning committee, or the Academies. The report offers a summary of the key issues and ideas identified during the symposium, but it offers no consensus conclusions or recommendations and is intended to reflect the discussions during the meeting. In order to be as responsive to the charge as possible, it is organized thematically rather than chronologically so that ideas raised at various points in the symposium are grouped together. A complete transcript that provides additional information about the contents of the presentations and discussions is available on the project website.[5]

OPENING REMARKS

The symposium was opened by Ralph J. Cicerone, president of the National Academy of Sciences (NAS). His remarks reflected on the long

[3] In addition to the summary report of the meeting, the archived webcast and the presentation slides may be found at http://dels.nas.edu/Workshop-Summary/Potential-Risks-Benefits-Gain/21666?bname=bls.

[4] The NSABB WG's paper, along with the commissioned papers, the archived webcast, and all the presentations at the January meeting, are available on the NSABB website at http://osp.od.nih.gov/office-biotechnology-activities/event/2016-01-07-130000-2016-01-08-220000/national-science-advisory-board-biosecurity-nsabb-meeting.

[5] The transcript may be found at http://dels.nas.edu/Upcoming-Event/Gain-Function-Research-Second/AUTO-9-61-70-Q?bname=bls.

BOX 1-1
Statement of Task

An ad hoc committee established by the National Research Council (NRC)[a] will organize two public symposia. The first symposium was held on December 15-16, 2014, and included discussion of the following topics:

- Principles important for, and key considerations in, the design of risk and benefit assessments of gain-of-function (GOF) research.
- Potential benefits of the research, including generating new scientific knowledge about viruses with pandemic potential, informing public health responses to a potential pandemic, supporting surveillance efforts to identify possible pandemic strains and provide more time for preparedness, and facilitating the development of vaccines and antiviral therapeutics.
- Potential risks associated with the research, in particular those related to biosafety and biosecurity.
- Alternative methods that may be employed to yield similar scientific insights and/or potential benefits, while reducing potential risks.

The second symposium—the focus of this task—to be held in early 2016, will focus on discussions of the National Science Advisory Board for Biosecurity (NSABB) draft recommendations regarding GOF research. This meeting will also include discussions of the results of the commissioned risk–benefit assessment as well as risk interpretation and analysis to inform decision making. This symposium will provide a mechanism to both engage the life sciences community as well as solicit feedback on optimal approaches to ensure effective federal oversight of GOF research. Of note, the public symposium should not include the development of consensus recommendations, but rather should elicit individual perspectives and robust discussion on the topics described above. Discussions at this symposium will inform the development of the NSABB's final recommendations.

The committee appointed by the NRC to organize and plan the second symposium will develop the symposium agenda, select and invite speakers and discussants, and moderate the discussions. Invited attendees should have a diverse range of perspectives and expertise, including but not limited to public health, biosafety, public health surveillance, research, risk assessment experts, public policy makers, security, and drug and vaccine development; the agenda should also include experts from regions of the world where pathogens with pandemic potential are endemic and from regions of the world conducting GOF research on such pathogens. This 2-day symposium will be webcast and the presentations and background materials will be archived online.

[a] On July 1, 2015, the institutional designation became the National Academies of Sciences, Engineering, and Medicine.
SOURCE: NIH, 2015.

history of the NAS's engagement with the complexities of balancing the risks and benefits of science and technology. Providing a neutral forum in which to discuss the scientific underpinnings of complex and controversial topics is one of the major missions of the Academies, and he urged participants to engage fully in the discussions over the two days of the symposium.

Margaret Hamburg, Foreign Secretary of the National Academy of Medicine, then discussed the evolution of oversight of so-called dual use research in the life sciences, from the 2004 report on *Biotechnology Research in an Age of Terrorism* to the current GOF discussions (NRC, 2004).[6] Dr. Hamburg highlighted the role of the Academies in providing science advice to government. She indicated the importance of the GOF debate and the international nature of the issues and diseases involved. Dr. Hamburg noted that while the discussions at the symposium were focused on advice for the U.S. government, they would have implications for the global research enterprise. She underscored the importance of the symposium and its role in building on a wide range of earlier discussions on policy frameworks and approaches to addressing GOF research. This meeting, according to Dr. Hamburg, was an opportunity to look at those frameworks and approaches and identify desirable next steps. She identified a need to develop a strategic approach to support scientific progress while addressing the impacts for our societies.

Jo Handelsman from the White House Office of Science and Technology Policy believed this to be a landmark meeting, one that could direct future policy in important ways. She noted that the White House has focused on issues around GOF research for 18 months and recognizes the need to keep life sciences vibrant but also to protect safety and security across the globe. Officials had become engaged because of concerns around the creation of new pathogens, especially those with pandemic potential. The White House has also worked to address safety incidents at laboratories that raised public concerns over work with such pathogens (Holdren and Monaco, 2014). In response to these concerns, in October 2014 the White House announced a deliberative process and, along with it, a pause on federal funding for certain types of GOF research (White House, 2014a). Dr. Handelsman highlighted the importance of key exceptions to the funding pause to enable necessary emergency research to continue.

The NSABB was asked to draft recommendations for a conceptual approach for dealing with GOF research that would then be made available for public comment. As mentioned above, the Academies were asked

[6] In this context, "dual use" refers to the dilemma that "the same technologies can be used legitimately for human betterment and misused for bioterrorism" (NRC, 2004: 1).

to convene two public meetings to facilitate a broad discussion of all the relevant issues: one to review technical developments, and a second to discuss the draft recommendations prepared by the NSABB as well as policy options for GOF. Dr Handelsman noted that the NSABB's draft recommendations would be revised in light of the discussions at this symposium and in line with the public input they have received. Following this, an interagency process led by the Office of Science and Technology Policy will produce a policy that will provide federal oversight for GOF research and replace the funding pause.

Carrie Wolinetz from the National Institutes of Health began her remarks by stating that a robust life sciences research endeavor is critical to promoting public health and well-being in light of evolving threats posed by microbial pathogens. This endeavor will entail a certain amount of risk, she noted, requiring a thoughtful approach to reducing risk while taking advantage of the broad range of benefits. She commented that GOF research was a fundamental scientific tool to:

- Help define the nature of host–pathogen interactions;
- Enable assessment of the pandemic potential of emerging infectious agents;
- Inform public health and preparedness efforts; and
- Further medical countermeasure development.

Dr. Wolinetz stated that some GOF experiments had raised safety and security concerns about whether they could result in engineered pathogens capable of causing a pandemic if accidentally or deliberately released. There was also concern that information describing their development could be used by those with malign intent to cause harm through a deliberate release.

Dr. Wolinetz described the GOF deliberative process (see Figure 1-1). She recalled that the deliberative process included a pause in funding for GOF research involving influenza viruses and Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV).[7] She highlighted the role that had been

[7] "New USG [U.S. government] funding will not be released for gain-of-function research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. The research funding pause would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity. In parallel, we will encourage the currently-funded USG and non-USG funded research community to join in adopting a voluntary pause on research that meets the stated definition" (White House, 2014a: 2).

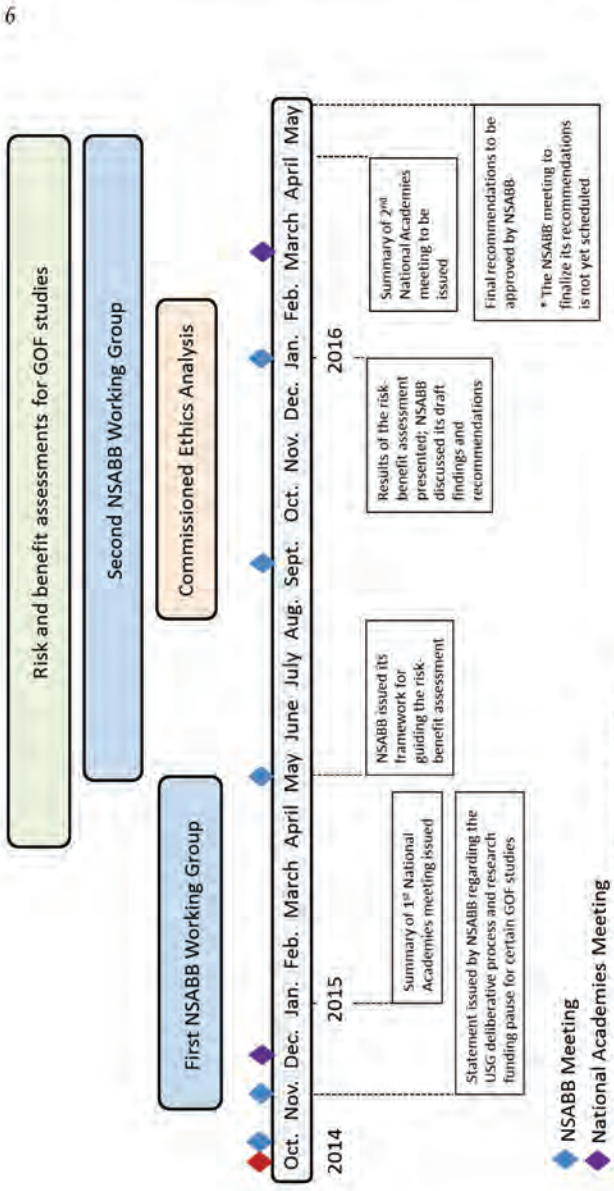

FIGURE 1-1 Timeline of major events in the gain-of-function deliberative process.
NOTE: GOF = gain of function; NSABB = National Science Advisory Board for Biosecurity; USG = U.S. government.
SOURCE: NSABB 2015a: 9.

played by the NSABB and recalled that it had been charged to advise on the design, development, and conduct of a risk–benefit assessment of GOF studies as well as to provide formal recommendations to the U.S. government on the conceptual approach to the evaluation of proposed GOF studies. During the process, the NSABB had also acted as a convening body. Dr. Wolinetz noted that the NSABB had received many valuable inputs to assist it in its work, including the report from the first Academies GOF symposium (NRC, 2015), the risk and benefit assessment conducted by Gryphon Scientific (Gryphon Scientific, 2015), and the ethics report commissioned from Michael Selgelid (Selgelid, 2015). Dr. Wolinetz concluded by noting that more input was being sought, for example, through the discussions at this symposium.

2

The Draft National Science Advisory Board for Biosecurity Policy Framework, the Risk and Benefit Assessment, and Insights for the Policy Process

OVERVIEW OF THE NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY DRAFT WORKING PAPER

Samuel Stanley, the chair of the National Science Advisory Board for Biosecurity (NSABB), highlighted the valuable role played by the National Academies of Sciences, Engineering, and Medicine's first symposium on gain-of-function (GOF) research in the deliberations of the NSABB's Working Group on GOF Issues (hereafter, NSABB WG), in particular during the development of its Draft Working Paper and recommendations. Dr. Stanley reviewed the activities undertaken by the NSABB since the start of the deliberative process. He reviewed the charge to the NSABB and highlighted the outputs produced to date, including *Framework for Conducting Risk and Benefit Assessments of Gain-of-Function Research* in May 2015 (NSABB, 2015b) and *Working Paper Prepared by the NSABB Working Group on Evaluating the Risks and Benefits of Gain-of-Function Studies to Formulate Policy Recommendations* in December 2015 (NSABB, 2015a).

Dr. Stanley introduced the NSABB WG's Draft Working Paper, noting that it included guiding principles for NSABB deliberations; analysis and interpretation of the formal risk and benefit assessment (RBA); consideration of ethical values and decision-making frameworks; analysis of the current policy landscape and potential policy options; preliminary findings from the NSABB WG's analyses; draft recommendations for the NSABB's consideration; and a number of important questions for further consideration. He reviewed key inputs into the work of the NSABB WG.

Dr. Stanley provided some reflections on the RBA prepared by Gryphon Scientific (Gryphon Scientific, 2015), describing it as rigorous and comprehensive and representing a monumental amount of work. The scope of the RBA addressed biosafety risks and biosecurity risks as well as benefits from GOF research. The study had allowed the NSABB to understand the different risks associated with research involving relevant pathogens and certain GOF experiments. It had helped them to identify and distinguish GOF studies that raise significant concerns from those that do not. Dr. Stanley indicated it assisted in identifying and evaluating the potential benefits of GOF studies and in comparing the potential benefits derived from GOF studies to those that may be achieved through alternative approaches.

Drawing on the ethics report prepared by Michael Selgelid (Selgelid, 2015), Dr. Stanley highlighted a number of important values to consider when evaluating research proposals involving GOF studies as well as when establishing mechanisms to review and/or make funding decisions about them. These included both substantive values (such as non-malfeasance, beneficence, social justice, respect for persons, scientific freedom, and responsible stewardship) and procedural values (such as public participation and democratic deliberation, accountability, and transparency).

He noted that there are multiple policies and frameworks already in place for managing risks during the research lifecycle (see Figure 2-1). These include reviews of the scientific merit of proposed research; measures for biosafety oversight, such as the *Biosafety in Microbiological and Biomedical Laboratories* manual (CDC and NIH, 2007) and the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH, 2013); the Federal Select Agent Program; the U.S. government policies for federal and institutional oversight of life sciences dual use research of concern (White House, 2012, 2014b); the Department of Health and Human Services framework for guiding funding decisions about certain GOF studies with highly pathogenic avian influenza (HHS, 2012); and measures that relate to sharing and communicating scientific findings and research products. Dr. Stanley noted that the success of these measures depends on effective compliance and implementation. He noted that there were different levels of oversight depending on what pathogen was involved and what was being done with it.

Dr. Stanley then summarized the key findings and recommendations from the NSABB WG's Draft Working Paper. The five key findings are:

1. There are many types of GOF studies and not all of them have the same level of risks. Only a small subset of GOF studies—GOF studies of concern—entail risks that are potentially significant enough to warrant additional oversight;
2. The U.S. government has effective policy frameworks in place for managing risks associated with life sciences research (see Figure 2-1).

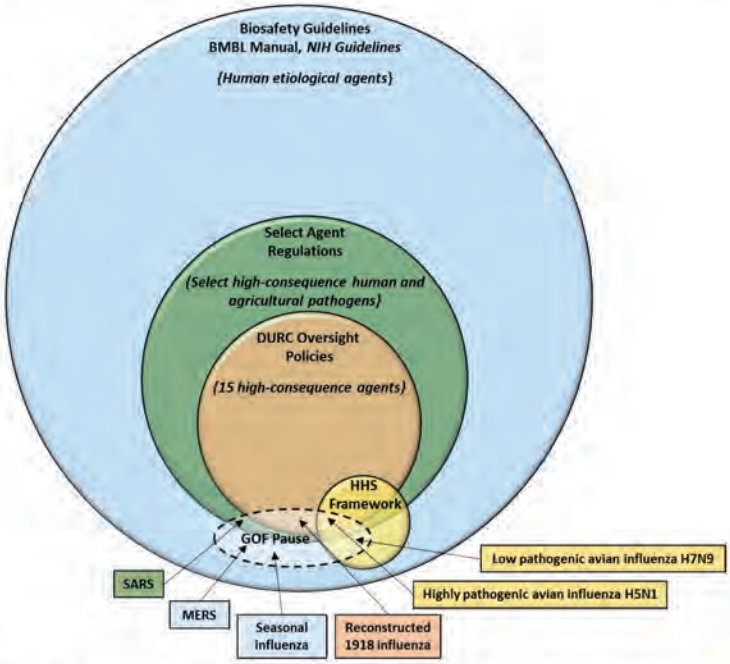

FIGURE 2-1 U.S. government policy frameworks for managing risks associated with life sciences research.
NOTE: BMBL = *Biosafety in Microbiological and Biomedical Laboratories*; DURC = dual use research of concern; GOF = gain-of-function; HHS = Department of Health and Human Services; MERS = Middle East respiratory syndrome; NIH = National Institutes of Health; SARS = severe acute respiratory syndrome.
SOURCE: National Science Advisory Board for Biosecurity, 2015a: 27.

There are several points throughout the research life cycle where, if the policies are implemented effectively, risks can be managed and oversight of GOF studies could be applied;

3. Oversight policies vary in scope and applicability, therefore, current oversight is not sufficient for all GOF studies that raise concern;
4. There are life sciences research studies that should not be conducted on ethical or public health grounds if the potential risks associated with the study are not justified by the potential benefits. Decisions about whether GOF research of concern should be permitted will

entail an assessment of the potential risks and anticipated benefits associated with the individual experiment in question. The scientific merit of a study is a central consideration during the review of proposed studies but other considerations and values are also important; and

5. The biosafety and biosecurity issues associated with GOF studies are similar to those issues associated with all high containment research, but a small subset of GOF studies have the potential to generate strains with high and potentially unknown risks. Managing risks associated with all high containment research requires Federal-level oversight, institutional awareness and compliance, and a commitment by all stakeholders to safety and security. Biosafety and biosecurity are international issues requiring global engagement. (NSABB, 2015a: 3-4)

The NSABB WG's Draft Working Paper also includes four recommendations:

1. Research proposals involving GOF studies of concern entail the greatest risks and should be reviewed carefully for biosafety and biosecurity implications, as well as potential benefits, prior to determining whether they are acceptable for funding. If funded, such projects should be subject to ongoing oversight at the federal and institutional levels;
2. In general, oversight mechanisms for GOF studies of concern should be incorporated into existing policy frameworks. The risks associated with some GOF research of concern can be identified and adequately managed by existing policy frameworks if those policies are implemented properly. However, the level of oversight provided by existing frameworks varies by pathogen. For some pathogens, existing oversight frameworks are robust and additional oversight mechanisms should generally not be required. For other pathogens, existing oversight frameworks are less robust and may require supplementation. All relevant policies should be implemented appropriately and enhanced when necessary to effectively manage risks;
3. The risk-benefit profile for GOF studies of concern may change over time and should be re-evaluated periodically to ensure that the risks associated with such research is adequately managed and the benefits are being realized.
4. The U.S. government should continue efforts to strengthen biosafety and biosecurity, which will foster a culture of responsibility that will support not only the safe conduct of GOF research of concern but of all research involving pathogens. (NSABB, 2015a: 4-5)

A key issue related to the first finding and recommendation is the question of what constitutes "GOF studies of concern." As Dr. Stanley explained:

> GOF research of concern would be a study that can be anticipated to generate a pathogen that is, one, highly transmissible in a relevant mammalian model, two, highly virulent in a relevant mammalian model, and three, is likely more capable of being spread among human populations than currently circulating strains of the pathogen. The first two characteristics are intended to involve the concept of the threshold. That is, the generated pathogen would need to be highly transmissible and highly virulent. Studies of pathogens with moderate virulence and transmissibility entail risks of course, but in general, those risks can be managed through existing mechanisms. The third criterion is intended to capture the concept of pandemic potential. That is, a pathogen could spread rapidly among human populations, either because there's no population immunity, no available counter-measures, or for some other reason. (Stanley, 2016)

The question of the appropriate criteria for defining GOF studies of concern was a recurring theme in subsequent discussions.

Dr. Stanley went on to explain that the NSABB WG had also identified a number of principles for guiding funding decisions related to GOF studies of concern (see Box 2-1).

To further assist in determining how such arrangements might function in practice, the NSABB WG had continued to develop the conceptual approach for the review, funding, and oversight of GOF studies of concern, including a new diagram (see Figure 2-2), which Dr. Stanley presented at the symposium. It includes activities to be undertaken at the institutional and federal levels and details what additional steps would be required for GOF studies of concern. He added that, as discussed at the NSABB's January meeting and in the NSABB WG's Draft Working Paper, the NSABB had highlighted a number of questions that needed further consideration and input (NSABB, 2015a: 46). He said that the NSABB WG was also considering a new question: "What type of body should be tasked with the high-level review of GOF research of concern. Would a FACA-like[1] committee be desirable, or as now envisioned by NSABB, can such reviews be accomplished by federal agencies, or other groups internal to the United States government?"

Dr. Stanley concluded by saying that the NSABB would continue working on its recommendations, with plans for a meeting scheduled for May 24, at which the final report would be discussed and possibly voted

[1] "The Federal Advisory Committee Act [FACA] was enacted in 1972 to ensure that advice by the various advisory committees formed over the years is objective and accessible to the public. The Act formalized a process for establishing, operating, overseeing, and terminating these advisory bodies" (General Services Administration, http://www.gsa.gov/portal/category/21242).

BOX 2-1
NSABB Principles to Guide Funding Decisions for Gain-of-Function Research of Concern

The following principles should guide the review of and funding decisions about research proposals anticipated to involve GOF research of concern:

i. The research proposal has been evaluated by a peer-review process and determined to be scientifically meritorious and has been assessed to be likely to exert a sustained, powerful influence on the research field(s) involved.
ii. An assessment of the overall potential risks and benefits associated with the project determines that the potential risks compared to the potential benefits are justified.
iii. There are no feasible, equally efficacious alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach.
iv. The investigator and institution proposing the research have the demonstrated capacity to carry it out safely and securely.
v. The research information is anticipated to be broadly and legally shared in order to realize its potential benefits to global health.
vi. The research will be supported through funding mechanisms that include appropriate oversight of (a) all aspects of the research including its conduct, (b) the sharing of data and materials, and (c) the communication of the research.
vii. The proposed research is ethically justifiable.

SOURCE: NSABB, 2015a: 43.

on. He encouraged the participants to continue to submit comments to the NSABB and to take an active role in the symposium discussions.

Harvey Fineberg, chair of the Symposium Planning Committee, then moderated an open discussion of the NSABB WG's Draft Working Paper. In his introductory remarks, Dr. Fineberg highlighted the importance of determining what does, or should, qualify as GOF studies of concern and the subset of research that may warrant additional oversight. He recalled the three characteristics identified by the NSABB WG and stressed the importance of defining the threshold between research of concern and other studies. He commented that the proposed definition did not take into account the starting point of virulence, transmissibility, or resistance of the pathogen. "If you have a very resistant organism that is very virulent if contracted, and all you want to do is to test whether the function of transmissibility could be enhanced, why would that be less of concern than starting with a less virulent, less resistant, less transmissible organism, and trying to produce increased function along all three

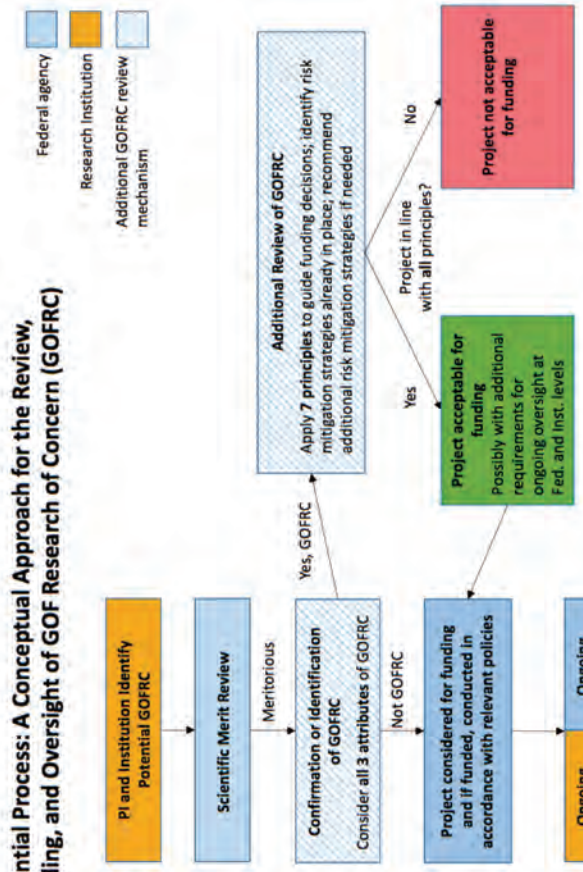

FIGURE 2-2 A conceptual approach for the review, funding, and oversight of gain-of-function research of concern.
NOTE: GOF = gain-of-function; PI = principal investigator.
SOURCE: Stanley, 2016.

dimensions?" He suggested that conceptually it might make more sense to think about "zones" of GOF research where concerns arise, because any combination of the three, or any one, two, or three, leads to a zone of concern outside of what the native organism represents (Fineberg, 2016).

Dr. Fineberg commented that the research enterprise is generally positive because it reveals truths of nature. But there could be a class of investigations that provoke scientific, ethical, or social concerns. In such cases, he felt, the burden of proof as to the value of a specific piece of research would move to those wanting to pursue it.

In conclusion, he added to Margaret Hamburg's comments, noting that, although the NSABB was focused on recommendations for U.S. policy, this was intrinsically a global challenge. He expressed the hope that during the symposium participants would consider the issues related to the development of a global regime to manage this class of research of concern, in addition to and beyond any national regime.

Discussion

Joseph Kanabrocki from the University of Chicago and Kenneth Berns from the University of Florida, co-chairs of the NSABB WG, joined Dr. Stanley on stage for the discussion.

The discussion that followed highlighted several themes from the presentations. The scope of assessing risks and benefits was explored, with questions raised by George Gao from the Chinese Academy of Sciences and the Chinese Center for Disease Control and Prevention about why there was comparatively little focus on loss-of-function experiments, given the difficulty of predicting which method would reduce, or increase, or enhance a virus's virulence or transmissibility. More broadly, Keiji Fukuda from the World Health Organization noted connections to other issues in which life sciences research lies at the heart of safety or security concerns, such as food security or genetically modified organisms. He highlighted the importance of engaging with access and benefit-sharing regimes.

Alternative approaches to tying increased oversight to funding were discussed. Some participants felt that direct regulatory approaches would be preferable. A review of different policy options from a recently published commentary was presented by Thomas Inglesby from the Center for Health Security, University of Pittsburgh Medical Center (UPMC), that included lifting the moratorium on GOF research; seeking an international consensus; securing national and international agreement to restrict the performance of GOF studies of concern; designating a board; establishing clear red lines for GOF studies of concern; and requiring the purchase by research institutions of specific liability insurance policies (Lipsitch et al., 2016). Megan Palmer from Stanford University reflected that several

of the questions identified by the NSABB WG as requiring further consideration corresponded to some of the tasks given to the NSABB by the White House. She also asked the NSABB WG to provide key lessons on the limitations of expertise or limitations in the process that might be fed into broader or future discussions on the oversight of life science research.

The importance of international collaboration was stressed and the potential for those wishing to undertake GOF studies of concern to relocate to less restrictive environments was noted by Abdulaziz Alagaili from King Saud University in Saudi Arabia. He also argued that any oversight frameworks should apply to the private sector as well as academia. Piers Millett from Biosecure suggested that an international component should be a significant aspect of future work, including the allocation of necessary resources; a mandate for long-term, sustained engagement; and genuine two-way conversations (rather than the presentation of a finalized solution). He also suggested that any international discussions should be co-hosted by relevant health and security entities to prevent perceptions of bias.

The outcomes from discussions held in other countries about GOF research were highlighted. Filippa Lentzos from King's College London, for example, in a comment made via the Web, noted findings from these discussions of the

- Lack of clear and convincing justifications for GOF studies of concern;
- Role of personal or institutional interests in agenda setting;
- Global dimension of GOF research of concern and the need for an international solution;
- Potential for accidents, abuse, and malpractice, and the intricate relationship between trust and accountability;
- Instability of political contexts and changing security environments, and the need for transparency in biodefense-related research; and
- Need for clear red lines on the most dangerous GOF experiments that apply to the public, private, and military sectors.

She also raised the issue of how to ensure that the lay public's voice is heard and incorporated into the decision-making process around GOF of concern research.

Another participant, Catherine Rhodes from the University of Cambridge, recalled a recent meeting in the United Kingdom in which influenza researchers indicated an interest in developing international approaches to the oversight of relevant research but feared any such process becoming dominated by existing U.S. policy discussions.

On the question of how to define "GOF studies of concern," some participants objected to requiring that all three of the characteristics recommended by the NSABB WG be met. Dr. Millett felt that the threshold for risk requiring additional oversight had been set too high and that any research that would be expected to produce a pathogen with any two of the characteristics should be considered for additional oversight. In this respect, Marc Lipsitch from Harvard University noted that the original GOF experiments that prompted the international controversy were initially believed to have met only two of these criteria, and eventually met only one. Thus, those experiments would not be subject to any of the oversight provisions under discussion. He and Dr. Inglesby argued that the third criterion was superfluous and that only issues of transmissibility and virulence need be considered. Dr. Lipsitch cited the original White House charge (White House, 2014a), public comments submitted to the NSABB by the Infectious Disease Society of America (IDSA, 2016), and "common sense" in support of his argument. John Steel from Emory University raised technical questions about how to measure these characteristics; for example, what does "highly" transmissible mean? He also cited the shortcomings of animal models to approximate transmissibility and called for additional guidance on how to make such decisions in practice.

The discussion also included a number of other specific reflections on the NSABB WG's Draft Working Paper and its recommendations

- Dr. Inglesby highlighted how important it was that any relevant regulations or other measures governing GOF studies of concern apply anywhere relevant research is being conducted, regardless of whether the laboratory receives federal funds or whether it is found in the public or private sectors.
- The value of the NSABB making its recommendations broad enough to fit GOF studies of concern with any pathogen, rather than just those covered by the moratorium, was noted by Dr. Millett. He also suggested that any characterization of GOF studies of concern should not be based upon taxonomy but instead focus on functional characteristics as contained in the draft definition.
- Dr. Selgelid from Monash University in Australia raised the possibilities of making oversight arrangements progress along a spectrum rather than being treated as binary. In such a model, a single risk threshold would not be established (above which research would be governed by specific oversight measures), but rather increasing levels of oversight would apply as the relative risk of the work increases. He also commented that, rather than first making a judgment about the scientific merit of a study and then assessing whether it raised GOF issues of concern, it might

be better to include considerations of risk at the earlier stage. If two studies show equal scientific merit and neither is considered of concern, then—other things being equal—would it be better to fund the less risky study if one cannot fund both?

- Questions as to the efficacy of the existing arrangement for addressing biosecurity information risks were raised by Dr. Inglesby and Dr. Millett, who encouraged further reflection on suitable oversight.
- Some participants, such as Dr. Millett, felt that bodies involved with assessing risks and benefits could not be housed within either the health or the security architecture but should be located inside a neutral agency.
- Questions about the interface between the proposed regulatory framework developed by the NSABB WG and existing arrangements for GOF experiments with specific agents, such as the one implemented by the National Institutes of Health (NIH), were raised by Nicholas Evans from the University of Pennsylvania. The ethics report also was discussed, with Dr. Evans suggesting that the scope of ethical issues related to GOF studies of concern was considerably broader than the scope of those included in the consideration of benefits in other areas, such as human subjects research. This, he suggested, would seem to increase the challenges in suitably reflecting potential benefits of GOF studies of concern.

Dr. Fineberg began the period of taking responses from the NSABB members by welcoming comments on any issue but said he hoped that they would reflect in particular on the "core question of what qualifies as being of concern." He noted that there had been a variety of viewpoints expressed about the necessity of meeting all three criteria, the implications of thresholds versus a spectrum, and the question he had raised earlier of whether the starting point could enough to make research meeting only one criterion "of concern." Dr. Kanabrocki responded by clarifying that the NSABB had not changed its thinking, as some had suggested, about the third criterion. The NSABB WG had recognized that there was some misinterpretation of the original language to limit the criteria to only resistance to countermeasures. Instead the intent is to capture the broader question of pandemic potential. Dr. Berns added that he emphasized "what's important is what you wind up with," that is, the potential for a pandemic and this led to the question of whether or not there were existing defense mechanisms. He also commented on the difficulty of predicting the consequences of research and the challenges in attempting to quantify such risks. Dr. Stanley commented that the issue was whether

the research could create risk significantly above the existing risk for that pathogen. Again, this tied to the questions of natural immunity or countermeasures.

In response to other questions and comments, Drs. Stanley, Kanabrocki, and Berns acknowledged the importance of the issues that had been raised, and they commented that the NSABB had struggled, for example, with the question of whether to offer broad recommendations or the more specific guidance for which some participants were calling. Dr. Berns commented that, even more than the definition, he thought the NSABB WG had struggled with the level at which the decision would be made. Should the final decision be made inside the government or by an outside group, such as a FACA committee? Efficiency might suggest handling the decision inside the government, but the public interest in transparency would argue strongly for a FACA committee. They also noted that, although the NSABB was tasked to make recommendations for the U.S. government, they recognized the importance of the international dimensions of the issue. Dr. Stanley thought all the members of the NSABB, as well as its sponsors, believed that it was necessary to "strive for a global solution here, and that some type of harmonization essentially of these processes would be extraordinarily valuable." The process was definitely not completed and they welcomed the input the NSABB WG would receive during the 2 days of the symposium.

LESSONS FROM THE RISK AND BENEFIT ASSESSMENT

Charles Haas from Drexel University, a member of the Symposium Planning Committee, introduced the goals of the session. The details of the risk and benefit assessment (RBA) had already been reviewed in detail at the January 2016 NSABB meeting.[2] The purpose was therefore to build on those prior discussions to consider how risk assessment more broadly could serve the important roles that the NSABB's draft findings and recommendations, including its proposed conceptual approach for making decisions about GOF studies of concern, had given to judgments about risks and benefits.

Rocco Casagrande from Gryphon Scientific provided an overview of the RBA (Gryphon Scientific, 2015) as basic background for the session. The purpose of this 8-month study was to provide data on the risks and benefits associated with research on modified strains of influenza viruses

[2] The webcast of those discussions, along with copies of presentation slides and written public comments, are available at http://osp.od.nih.gov/office-biotechnology-activities/event/2016-01-07-130000-2016-01-08-220000/national-science-advisory-board-biosecurity-nsabb-meeting.

and the coronaviruses. The RBA had been divided into three major tasks, each of which required a distinct data collection and analysis approach: a quantitative biosafety risk assessment; a semi-quantitative biosecurity risk assessment; and a qualitative benefit assessment. Dr. Casagrande noted that the RBA was comparative; it determined the change in risk from research on GOF pathogens compared to research on wild type pathogens and identified the benefits to science, public health, and medicine afforded by GOF research compared to alternative research and innovations.

Dr. Casagrande presented key findings from the RBA. The biosafety risk assessment included a map of the series of events necessary for a laboratory incident to result in a pandemic. The probability of each event resulting in the next necessary event was determined. The RBA established that only a small minority of laboratory incidents with the most contagious influenza viruses would cause a local outbreak, and only a minority of those would lead to a global pandemic.

The published RBA had identified the pandemic strain of the 1918 H1N1 influenza virus as posing the greatest risk. However, subsequent information made available to Gryphon Scientific at the January NSABB meeting by Dr. Kanta Subbarao from NIH showing a high degree of cross protection afforded by exposure to the 2009 influenza against the 1918 influenza enabled a reassessment. Further analysis determined that the naturally circulating 1957 H2N2 influenza virus became the "riskiest" pandemic strain because its antigenic profiles would cause about 100 times more global cases, although it is only one-tenth as deadly as the 1918 strain.[3] As a result, it became the comparator against which other risks should be evaluated. The RBA also determined that the riskiest modified strain was a 1918 H1N1 strain altered to evade residual immunity or to be otherwise more transmissible.

Other key findings from the biosafety assessment included

- Manipulating GOF seasonal influenza strains at the BSL3 level may compensate for the increase in risk posed by the modified strains, largely because the extra system of respiratory protection decreases the risk of a laboratory acquired infection.
- Some of the manipulations that could theoretically increase risk may not be achievable or desirable. For example: (i) a strain that can overcome protective vaccination increases risk only if it can evade vaccine protection via immune modulation, not antigenic

[3] The details of the Gryphon Scientific analysis are available in supplemental material on its website: http://www.gryphonscientific.com/wp-content/uploads/2016/03/Supplemental-info%E2%80%93Protection-against-Infection-with-1918-H1N1-Pandemic-Strain.pdf. The final version of the report was released in April 2016 (Gryphon Scientific, 2016).

change; (ii) the scientific value of increasing the transmissibility of influenza virus beyond that of the most transmissible strains (or final titer beyond 1E8) is questionable and perhaps infeasible; (iii) there is no animal model of transmission for the coronaviruses, so manipulation of this trait is not currently achievable; and (iv) some estimates suggest that severe acute respiratory syndrome coronavirus (SARS-CoV) may already be more transmissible than was estimated in the RBA, in which case further manipulation would not affect risk.

The biosecurity risk assessment had two components: an assessment of the risks from acts targeting a laboratory; and security risks derived from the information generated by the studies. Key findings of the assessment of risks from acts targeting a laboratory included

- The traits that drive risk are similar when considering biosafety and biosecurity because the pathogens are transmissible. How the initial infections were caused is of little consequence once a local outbreak begins.
- Biosecurity events are often predicted to involve the covert infection of the public, so this type of an infection is much more likely to cause a global outbreak. By contrast, laboratory workers benefit from health surveillance and isolation protocols not available to the general public.
- To match the risk posed by biosafety incidents given a historical rate of laboratory acquired infections, a biosecurity event that covertly infects a member of the public must occur only once every 50-200 years. These events include theft of an infected animal, contaminated piece of equipment, or viral stock. Given the frequency with which these biosecurity events have happened, the RBA suggested that biosecurity be given as much consideration as biosafety.

The information biosecurity risk assessment analyzed "the risk that a malicious actor might misuse the information in publications describing GoF research" (Gryphon Scientific, 2015: 212). Key findings included

- Minimal information risk remains for GOF studies in influenza viruses because dual use methods have already been published.
- Significant information risk remains for GOF studies in the coronaviruses, but these studies are hampered by a lack of model systems.
- Information risk could easily be generated by research on other transmissible pathogens.

The benefits assessment identified GOF studies providing critical or unique benefits for both

- Influenza viruses, including studies that enhance viral growth from low titer; lead to evasion of residual or induced immunity; enhance virulence; enhance transmissibility; and lead to evasion of therapeutics in use and in development. And,
- Coronaviruses, including studies that alter host tropism; enhance virulence; and lead to evasion of therapeutics in development.

Dr. Casagrande highlighted a number of lessons learned during the execution of the RBA. He stated that the distinction between seasonal and pandemic flu is artificial because an old seasonal flu strain could become a new pandemic strain (as highlighted by 1957 H2N2 replacing 1918 H1N1 as the riskiest pandemic strain). He noted the lack of data on human reliability in life sciences laboratories in contrast to data from other well-researched sources such as the nuclear, chemical, and transportation industries. Those data show that human error is the most common cause of accidents. To use an example from the life sciences, it is more common for a lab worker not to use a powered air purifying respirator (PAPR) properly than for a PAPR to be defective. He also cited the difficulty posed by having no risk benchmark for work with wild type pathogens and the difficulty posed by restriction of the RBA to influenza and coronaviruses.

The RBA was then applied to a number of specific experiments, including those that

- Include virulence factors from 1918 H1N1 influenza in a 2009 H1N1 strain, which did not increase the probability that an outbreak escapes local control and indicated that global consequences scale linearly with case fatality rate.
- Aim to create antigenically distinct strains of a recently circulating seasonal influenza strain, which resulted in strains having a 2-3-fold increase in risk of escape, capable of inflicting 10 times more global deaths, resulting in a 20-30-fold increase in risk of infection. The meaning of this risk increase is difficult to interpret in the absence of standards for risk tolerance but suggests that more controls and measures should be taken to control infection risk from this modified pathogen than from the wild-type pathogen.

Dr. Casagrande also noted that bench researchers may not be familiar enough with the epidemiological properties of pathogens to properly characterize their strains. Guides or tools are needed to easily obtain

parameter values for wild type strains and, perhaps, to aid with the calculations.

A series of commentators provided reflections on the RBA. They were asked to consider

- What they know needs to occur, based on their prior experience in the context of policy making, to make use of the Gryphon Scientific analysis and other information.
- The potential value of risk–benefit analysis in making decisions on individual cases of proposed research projects rather than the role of a study intended to cover an entire class (i.e., GOF) of investigation, which was the purpose of the Gryphon Scientific analysis.

Louis (Tony) Cox from Cox Associates highlighted the value of attempts to quantify risk in the RBA (Cox, 2016). Dr. Cox also discussed risk management, or what to do about that risk, especially as it related to determining which proposals to fund. Dr. Cox highlighted the value of clearly defined decision rules and conditional decision rules, detailing the conditions that would need to be met before a proposal might be funded. Dr. Cox reflected that efforts to determine the maximum acceptable risk were not useful approaches in a GOF setting. He argued that both the context and the benefits needed to be taken into account and suggested that attempting to improve the risk–benefit profile may be a more suitable approach. Dr. Cox suggested that "arbitrary coherence"—accepting risks because they are less risky than those we already accept—was also not appropriate in a GOF context. He believed that benchmarks and precedents were not necessarily the most appropriate basis for decision making but supported gathering more information before making funding decisions, including on opportunity costs. He asserted that there is a need to learn from past experience and to make the decision-making process adaptive. Dr. Cox also identified a series of specific proposals for strengthening funding decisions on GOF studies of concern (see Box 2-2).

Kara Morgan from Battelle Memorial Institute reminded participants of the difficulty of decision making on low-probability, high-risk events. Dr. Morgan introduced a number of tools developed to assist in such situations and help match decision-making complexity to potential risk. She discussed three frameworks for decision making, describing the frameworks as part of a continuum, enabling their adaptation to different contexts (see Box 2-3).

Dr. Morgan concluded that decision making is a social process, not an analytical one. There is a need for a process to help move from analysis to a decision. She advised the symposium that decision frameworks, rules, and process were just as important as the analysis.

BOX 2-2
Proposals for Improved Funding Decision Making on Gain-of-Function Research of Concern

- A decision rule maps available information to decisions—specifically whether to fund, not to fund, to require modifications before funding, or to seek additional information on which to base a funding decision.
- The performance of a decision rule can be evaluated for a stream of simulated projects with specified risk, cost, benefit, and information/uncertainty characteristics and proposer response characteristics.
- If we know enough about GOF research to simulate realistic project proposals and decision rule performance, then simulation-optimization of decision rules can lead to better (higher-performing) individual project-funding decisions.
- Otherwise, eliminate dominated decision rules (e.g., risk matrices, simple additive scoring systems)

SOURCE: Cox, 2016.

Adam Finkel from the University of Pennsylvania set out five factors to strengthen risk–benefit analysis that should be integrated into the development of the policy framework for GOF studies of concern (see Box 2-4).

Dr. Finkel noted considerable differences in opinion among different risk estimates of GOF studies of concern. He argued that risk was not a binary state and this provided the potential for a hierarchy of decision rules. He also noted the importance of including justice and equity for those individuals affected by risk. Dr. Finkel commented that it becomes much harder to assess risk when uncertainties exist and they are uncorrelated. He also felt that it was necessary to do a better job of communicating the benefits of GOF research. He called for further efforts to identify where the faults that lead to risk are occurring. He introduced a new study of existing best practices in regulatory excellence based on the concept of "listening, learning, and leading" developed through work in the Canadian energy sector (Coglianese, 2015).

Dr. Finkel discussed the importance of basic laboratory safety. He believed the best way to prevent accidents from infecting the population was to prevent them from infecting laboratory workers. Dr. Finkel concluded by encouraging the use of a more solution-focused risk–benefit analysis—where options are not restricted to a specific limited set of options—but one which focuses on the underlying policy need. He provided examples from sources of drinking water and synthetic biology. He cautioned that uncertainties rarely cancelled each other out in practice.

BOX 2-3
Different Models for Risk-Based Decision Making

Acceptable risk—In this model the risk is estimated along a spectrum. A boundary or threshold is set above which one decision would be taken and below which a different decision would be taken. The main challenge with this approach is derived from the innate uncertainty of science. While it is possible to mitigate this through the use of safety factors, it can still result with benefits not being taken into account. The process of determining where the boundary falls can also be challenging and past discussions on GOF have already demonstrated notable differences of opinion on this point.

Risk–benefit assessment—This is a two-factor analysis and builds on an understanding that societies are often prepared to tolerate some risk if they receive benefits in return. While this model does take benefits into account, to be fully effective, it is necessary to express both risks and benefits in comparable terms, preferably using the same metrics. This can often involve value judgments. A decision as to where the appropriate balance lies between risks and benefits is often subjective.

Deliberative criteria-based frameworks—This model allows the introduction of more factors. It enables the integration of different views through the use of criteria identified in advance of assessment. It can include both scientific contexts based upon observations and perceptions (such as facts, data, analytical results, assumptions, and uncertainties) and social contexts based on values (such as goals, objectives, priorities, concerns, ethical issues, non-observable criteria, policy decisions, and tradeoffs). This model is more resource intensive than the other approaches but is more collaborative.

SOURCE: Morgan, 2016.

BOX 2-4
Factors for Strengthening Risk–Benefit Analysis

1. Risk and benefit estimates should be balanced, quantitative, humble, explicit about value judgments, and channeled in service of a thoughtful decision rule.
2. Benefit estimates can be made commensurable with risk estimates, and should be communicated with equal care.
3. Purely risk-based prioritization is inferior to net-benefit prioritization.
4. Transparency in public engagement is important, but not as important as "apparency" (which provides information on rationale and motivations).
5. "Solution-focused" analysis of GOF and public choice may require wholly new institutional arrangements, not just incorporation into existing policy frameworks.

SOURCE: Finkel, 2016.

Discussion

The resulting discussion began by highlighting the importance of having good baseline data against which to measure risk: for example, through a national database or framework of laboratory near misses, accidents, or disclosures, as discussed by James Welch from the Elizabeth R. Griffin Foundation. Panelists noted that the U.S. government had already committed to develop such a database (U.S. Government, 2015: 4).[4] The need for additional resources to undertake focused research to fill data gaps was highlighted by Gigi Kwik Gronvall from the UPMC Center for Health Security. Shortages of data on benefits and risks were felt by several participants to apply to infectious disease research and emerging areas of life science research more broadly. The importance of tools to enable scientists to operate safely and securely on an ongoing basis was also noted by Dr. Gronvall. There was also a discussion, prompted by Allison Mistry from Gryphon Scientific, of the need to differentiate between conducting functional changes in wild type as opposed to research backbones or chassis. The value of including comparative risks in different chassis in definitions of GOF and GOF studies of concern was also explored.

Participants also considered the limits of comparing risks and benefits in this type of research. The discussion explored the challenges in suitably reflecting the potential public health benefits of research. Corey Meyer from Gryphon Scientific, who had led the benefits assessment portion of the RBA, made a number of comments. She said that although it may be possible, at least qualitatively, to compare the risks and benefits of research for public health, she was not sure that was true for the benefits of scientific knowledge. She also wanted to underscore that "while the risks of the research are immediate in that they are occurring at the time the research is being conducted, the benefits to public health will be realized in the future. And there is significant uncertainty in how long it will take for those benefits to be realized because translation of basic science research into public health benefits is complex and depends on many other factors."

Adam Finkel commented that there is a substantial literature on discounting and the time value of benefits on which one could draw. He thought the problem was not intractable and offered the example of climate change research, where he said there is a movement toward lower discount rates. In this area, "the future speaks more loudly than we

[4] The recommendation—"Establish a new voluntary, anonymous, non-punitive incident-reporting system for research laboratories that would ensure the protection of sensitive and private information, as necessary"—is one of the products of the Federal Experts Security Advisory Panel, whose report was made public in October 2015 (U.S. Government, 2015).

have allowed in the past." So that part is not at all intractable. He cited the example from Michael Selgelid's white paper of benefits in terms of expected lives saved.

Rocco Casagrande commented that the daunting part of assessing future benefits is not how much to discount potential lives saved but how to make an estimate of how likely it is that any scientific discovery will lead to a public health benefit. Tony Cox commented that it also depends on what other research is done. And Kara Morgan noted that even failed experiments may offer useful lessons. John Steel also noted that such research can help ameliorate disease events that happen infrequently but that potentially result in tens of millions of deaths.

Some participants, such as Marc Lipsitch, questioned the findings on the unique benefits of certain GOF research, suggesting that the knowledge could have been generated using alternative approaches. In his view, the net contribution of GOF research to knowledge on influenza viruses has been overstated. He also said that the knowledge about mutations and phenotypes identified by Fouchier and Kawaoka had already been identified in previous safe experiments. The confirmation that they were important in the GOF context was new, but he asserted that their utility for public health prediction was so far unproven. "So the net benefit for public health is much smaller than the net knowledge." Issues around identifying and ensuring sufficient oversight of dual use research more broadly were also discussed, including that, as life science and biotechnology tools are getting more powerful, the potential for their misuse for malign purposes might also increase. On the RBA, some participants, such as Piers Millett, reflected that the process of updating the risk comparator from the 1918 influenza strain to the H2N2 1957 pandemic strain was a practical example of the importance of the inclusion of the concepts of innate or acquired immunity against pathogens in the third set of characteristics proposed by the NSABB to define GOF studies of concern. He also suggested that the RBA was a missed opportunity to explore the international opportunity costs associated with different decisions on GOF studies of concern, from a moratorium on relevant research, through increased oversight, to taking no additional steps.

The shortcoming of existing arrangements in identifying and mitigating biosecurity information risks was noted by some participants, including Victoriya Krakovna from the Future of Life Institute, Dr. Millett, and Megan Palmer. They argued that these assessed risks were only low because the critical information had already been released into the public domain over the last decade. This led to questions about the efficacy of current arrangements to identify potential future biosecurity information risks, such as those for coronaviruses highlighted in the RBA. The value of encouraging comments and reflections on the RBA and associated

methodologies from a wider group including different types of expertise was also noted by Megan Palmer.

THE SCIENCE OF SAFETY AND THE SCIENCE OF PUBLIC CONSULTATION

Baruch Fischhoff from Carnegie Mellon University, a member of the Symposium Planning Committee, opened the session by explaining that there had been a successful session at the first Academies GOF symposium, which offered an introduction to the lessons from research into human factors, public consultation, and risk assessment to inform the preparations by NIH and the NSABB for the RBA. This year the planning committee had organized another session focused on the insights that social science research can offer about the design and implementation of federal oversight for GOF studies of concern. The panel included experts in organizational culture, human factors, and public consultation who would offer comments on the NSABB draft recommendations and specific suggestions for the ultimate choices to be made by the U.S. government.

Ruthanne Huising of McGill University introduced the insights about compliance with safety regulations in life science laboratories gained from past research in which she had taken part (see, e.g., Huising and Silbey, 2011). Since 2012 she had also been observing Canadian regulators design new biosafety and security regulations that went into force in December 2015 using an intensive public consultation process.

Dr. Huising discussed behavior and decision making as mediated by social organizations, which can include both formal social structures (such as organizations and families) and what she termed emergent systems of meaning ("culture"), which include norms, values, and assumptions. The incidents that led to the GOF deliberative process had provoked extensive discussions of the existing culture of life sciences laboratories as this affected safety and security. In these and similar discussions, the concept of culture is often treated as both the "problem" (a "lax culture" or "insufficient culture") and the "solution" ("build a culture of safety," "change the culture"). Culture, she argued, is often understood as a managerial tool. She described how concepts of culture can be applied to understand how laboratories approach and implement safety provisions.

Dr. Huising described how culture might be shaped through socialization processes. Beginning with graduate training, researchers are observing and learning how successful members of their field think, talk, and act. They learn how competent, respected members of the community behave, potentially through their attention to safety, security, and risk.

Safety cultures can be designed and Dr. Huising provided examples of the systems used by BP and Dow Chemicals. Such efforts tend to be

top down and centralized, she noted. They can be slow to develop and expensive, and they often ignore differences in interests and resources. She suggested that safety cultures can also emerge, resulting in multiple, heterogeneous cultures. Such change often occurs in response to shocks, with new values and norms emerging. This approach can be slow, but it is self-reproducing. Dr. Huising felt this model might be more suitable for the scientific endeavor, in part because it would better reflect the nature of the organizational structure of research laboratories.

The organizational structure of relevant institutions can also impact culture, Dr. Huising noted, with administrative and academic laboratory components operating with different logics. Academic administration is organized in ways that give it considerable similarities to the organization of regulatory and other government agencies. In contrast, the laboratories, at least in theory, operate through collegial governance and a democratic approach to organizing. That said, principal investigators (PIs) have remarkable autonomy in how they organize and run their laboratories. Dr. Huising commented that "Decision making is highly decentralized, and often operates according to verbal agreements. Trust is a very important component in how things get done in these laboratories." Because the laboratories work on soft money, they are often in flux, with continuously changing resources, members, and activities. She also noted that these different professional bureaucracies have implications for biosafety and biosecurity, in particular by determining responsibility for legal and administrative requirements, allocating, authority to enforce those requirements, and facilitating compliance.

Dr. Huising presented key findings from studies of safety culture in biology laboratories. She emphasized that these studies came from BSL2 facilities because of the difficulties in obtaining sustained access to higher containment (BSL3, BSL4) facilities. The findings include:

- Researchers experience compliance requests as intrusions and impediments to their work. They communicate safety as peripheral to their research work and sometimes delegate it to students. They are most likely to incorporate safety features into their practices when they align with efforts to control physical materials.
- Most violations are minor (housekeeping). A small number of laboratories account for the majority of violations.
- Organizations depend on environmental, health, and safety (EHS) staff (such as Biosafety Officers) to ensure compliance.

With regard to the last finding, she noted that the roles of the EHS staff included buffering researchers from record-keeping, inspections, corrections, and helping to maintain compliance. They negotiate increased

daily compliance by working in laboratories, generating familiarity, trust, and relationships, which also gave them the ability to anticipate problems and to identify emerging dangers. In many cases, the EHS staff was able to draw on requirements and regulations to increase their resources and authority in relation to faculty, but she commented that these "boots on the ground" were chronically underfunded.

She noted the emergence of a "responsibility movement" in other facets of the life sciences, with examples of good practice in green chemistry, nanotechnology, synthetic biology, and the citizen science movement. Dr. Huising concluded by providing a number of specific recommendations for developing policy options for GOF research (see Box 2-5).

Gavin Huntley-Fenner from Huntley-Fenner Advisors introduced concepts in human factor research relevant to the NSABB WG's Draft Working Paper and recommendations. Dr. Huntley-Fenner highlighted that, in general, human error has increased proportionally as a contributor to accidents. He recalled that for laboratory biosafety, despite advances in technology, instruments, and personal protective equipment, the World Health Organization had asserted that "human error remains one of the most important factors at the origin of accidents" (WHO, 2006). He noted that there was a lack of data on human reliability in laboratories, and he stressed the importance of collecting more data on safety. He cited the conclusion in the RBA that "The state of knowledge of the rates and consequences of human errors in life science laboratories is too poor to develop robust predictions of the absolute frequency with which laboratory accidents will lead to laboratory acquired infections" (Gryphon Scientific, 2015: 3) to underscore the relevance for GOF policy deliberations.

BOX 2-5
Shaping Cultural Change Relevant to the Oversight of Gain-of-Function Research of Concern

- Culture change should come from within the scientific professions, making it more likely to produce long-term, global changes.
- Particular focus should be placed on the roles of Biosafety Officers in relevant laboratories, and that will require resources and support.
- Research about daily decisions and practices in laboratories needs to be supported and expanded to encompass higher containment facilities, providing for better baseline data.

SOURCE: Huising, 2016.

There are a range of factors that can contribute to the emergence of error, including how physical and cognitive stresses undermine human reliability, according to Dr. Huntley-Fenner. He suggested that the comparative scarcity of accidents might still mask latent risks, with more numerous incidents and errors going unreported. He highlighted research by the Government Accountability Office in 2009 that concluded latent risks still exist in laboratories from underappreciated human error (GAO, 2009). He suggested that human factors research could provide tools for designing, implementing and maintaining systems in which errors are mitigated when they occur. The benefits of incorporating human factor principles were potentially significant, with Dr. Huntley-Fenner suggesting that they could reduce risk associated with GOF studies of concern substantially. He noted that some simple approaches could yield a significant reduction of errors, such as the development and use of simple checklists, which had a significant impact in reducing surgical errors in hospitals in both developed and resource poor countries (Haynes et al., 2009).

Progress has been made in other areas to address shortcomings in human factor safety data. For example, Dr. Huntley-Fenner discussed how the National Aeronautics and Space Administration has succeeded in mining data it already had in ways that provided insights into areas where it had less data, which was then used successfully to reduce risk (Chandler et al., 2010). He argued that limited relevant laboratory safety data do exist. For example, a survey of laboratory acquired infections in 68 institutions in Belgium indicated that 95 percent of the incidents involved human error (Willemarck et al., 2012: 14). He suggested that the human factors research community was well positioned to provide relevant data but more work was needed in high-containment laboratories.

Measuring incidents was only one necessary step; controlling incidents was also important, noted Dr. Huntley-Fenner. He highlighted research that showed the success of applying multifaceted controls. He also highlighted the importance of considering context. Guidance from the United Kingdom on human factors that result in noncompliance with standard operating procedures demonstrated that cutting corners was mainly "due to situational and organizational factors. These factors include, for example, time pressure, workload, staffing levels, training, supervision, and availability of resources" (Bates and Holroyd, 2012). Dr. Huntley-Fenner recommended rigorous data collection and sophisticated analytics to reduce risk associated with GOF studies of concern (see Box 2-6). The self-driving Google car was provided as an example of successfully gathering and leveraging data on human decision making and error to build a system that reduces those risks.

Monica Schoch-Spana from the UPMC Center for Health Security outlined four basic considerations for the design of public deliberations.

BOX 2-6
Improving Rigorous Data Collection and Sophisticated Analytics to Reduce Risk Associated with Gain-of-Function Research of Concern

- Create national reporting standards that go beyond the most significant adverse events
- Collect data on near misses
- Collect data across multiple bodies to counteract relative rarity of events
- Standardize data inputs whenever possible
- Develop analytics driven models of when and what adverse events are more likely to occur and under what circumstances
- Direct training and other interventions where they are needed most

SOURCE: Huntley-Fenner, 2016.

1. **Which public(s) to involve in deliberations**—In the context of GOF studies of concern, Dr. Schoch-Spana suggested that considering three overlapping categories would be useful: the *pure public*, or naive citizens; the *affected public*, or persons or groups whose lives are altered or influenced by a policy decision; and the *partisan public*, or representatives of groups with vested interests or expertise in the policy matter. She also noted that each of these categories of the public had been engaged in past discussions on GOF studies of concern, with the affected public implied in the RBA, affected publics and the pure public noted in the ethics analysis, and partisan publics reflected in relevant publications and comments.
2. **What is the purpose for public(s) deliberation**—Three distinct aims were highlighted: *knowledge exchange*, conveying information from policy makers to publics, or transmitting views, opinions, or attitudes from the publics to policy makers; *innovation*, eliciting rich unpredictable insights that come from crowd-sourcing a problem or from experiential, on-the-ground knowledge; and *democratic accountability*, ensuring broad representation in a decision about the common good. If the public deliberation on GOF studies of concern was intended for democratic accountability, Dr. Schoch-Spana noted it was necessary to give people the time, information, space, and authority they need to perform that role. Merely bringing "ordinary people" or a cross-section of society together to deliberate does not automatically achieve this aim.

She suggested a series of desirable characteristics for public deliberations on GOF studies of concern, including diversity, balance, civility, accountability, and consent.

3. **Which process enables the public to fulfill its purpose**—The use of three types of processes in the GOF deliberative process were reviewed: *communication*, a form of transparency through putting out information for the public—for example, press releases, educational websites, and summary reports such as those made available by the first Academies GOF symposium and the NSABB GOF meetings, as well as making the RBA available online; *consultation*, a means of gathering input, such as through enabling public comments on draft NSABB recommendations and to the U.S. government on future funding and oversight policy; and *collaboration*, a more deliberative option to exchange ideas and share responsibility for making and implementing policy. To date, she felt that the life sciences and other partisan publics have had strong input but deliberation with the broad public has not yet been explored.
4. **On what problem will the public(s) deliberate**—Dr. Schoch-Spana reviewed good practices in identifying problems, especially where there are conflicting values as to the public good, and for controversial and divisive topics. She used them to identify three questions on which the publics might deliberate for GOF studies of concern: (i) Despite potential contributions to public health, should studies that could produce a pathogen of pandemic potential be performed at all?; (ii) Are finite dollars better spent on experiments to create pathogens with pandemic potential (which produce unique knowledge) or on strengthening the rest of the flu preparedness portfolio?; and (iii) If any, what added steps should trustee institutions (e.g., the U.S. government or research entities) take to strengthen pathogen of pandemic potential biosafety and biosecurity protections and public confidence in them?

Dr. Schoch-Spana also discussed how to operationalize standard elements of deliberation design. She noted that there is no single methodology for public deliberation, but she did describe a number of minimum standards for public deliberation, in particular for inclusivity and diversity, the provision of information, value-based reasoning. She also discussed methods for measuring the success of the process. Dr. Schoch-Spana concluded that meetings to date have engaged individuals from the life sciences, security, public health, biosafety, risk analysis, and the drug and vaccine industries, but the general public had been largely absent. She identified an unresolved issue of whether more sophisticated, resource-intensive deliberative sessions could be held outside the present circle

of vested parties. A number of possible activities for such a process were suggested (see Box 2-7).

In opening the floor for discussion, Baruch Fischhoff commented that the panelists had been encouraged to offer recommendations based on their own professional experience and research. He added that for those in the audience who were not familiar with the social, behavioral, and decision sciences, the panel should have provided some idea of the breadth and depth of the research that is available if one wanted to put the human aspect of this enterprise on a scientific foundation. It also illustrated the mix of methods used in this research: various theories; multiple methods of observation, including direct observation and laboratory and field experiments; traditional and statistical and analysis; and various types of data.

Discussion

The resulting discussion further elucidated specific aspects of the presentations. The importance of additional data gathering on accidents and associated human factors research was a repeated theme. Susan Wolf, an NSABB member from the University of Minnesota speaking in her personal capacity, raised operational issues around data collection, data standards, and the development of data collection systems. Gavin Huntley-Fenner commented that the dearth of current data on accidents and human reliability in laboratories does not mean that what people want to know is unknowable. He and others also noted the value as well as the potential challenges in implementing confidential accident reporting. The need to ensure that comprehensive reporting systems for

BOX 2-7
Examples of More Sophisticated, Resource-Intensive Deliberative Activities to Extend the Current Process

- Initiate a formal evaluation process to determine how the (primarily) partisan publics rate the quality of deliberations in terms of inclusivity, information provision, and value-based reasoning.
- Hold deliberative exercises in communities now hosting facilities where GOF studies of concern are undertaken.
- Engage a cross-section of the American public in a deliberative exercise about a specific question.

SOURCE: Schoch-Spana, 2016.

human errors are developed and implemented in a nonpunitive manner was stressed by Kavita Berger from Gryphon Scientific. In response, Dr. Huntley-Fenner noted the importance of even seemingly small things, such as finding language for reporting forms that did not use negative categories ("theft," "loss"), and designing systems so that there was feedback or other incentives for reporting, such as providing information that could be used to improve safety. Ruthanne Huising said that the new regulatory framework developed in Canada had a nonpunitive reporting system that offered potential lessons about dealing with privacy issues and offering useful feedback.

Given what he saw as the difficulties of implementing a nonpunitive system in high-containment laboratories, Andrew Kilianski, a National Research Council Fellow from the Edgewood Chemical Biological Center Aberdeen Proving Ground, made the specific suggestion to conduct research focused on the possible relationships between human error by graduate students under minimal-containment standards and other indicators of their proficiency. Megan Palmer from Stanford University highlighted the importance of strategic interventions to allow sustained scholarship on the social and behavioral dimensions of research. Monica Schoch-Spana commented that best practices for biosafety and biosecurity have not been captured, synthesized, and disseminated by researchers.

Adam Finkel said he was concerned that there had not been a discussion of a confidential channel for reporting incidents, citing what he thought was becoming a less favorable climate for "whistle-blowers" in many settings. He also stressed the need to consider outside incentives to support a culture, including enforcement. He thought that traditional regulation was probably not appropriate but cited other models, such as third-party audits, that could be considered.

Issues around the enforcement of safety and security regimes were also addressed, with some participants noting that a subsection of accidents and incidents are a result of negligence and malfeasance, requiring some form of censure. These individuals highlighted the importance of access to necessary resources for enforcement.

Opportunities for strengthening safety by designing out the consequences for human error were also noted: for example, by Dr. Finkel. He cited useful precedents from health care settings and commented that he sensed opportunities were not being widely studied or implemented in laboratory settings. In response, Dr. Huntley-Fenner noted a paradox that, as one designs out the other sources of error, human factors become an increasing portion of whatever error remains. That is not a reason to neglect those helpful improvements, but it is a reminder that human error will always be with us.

Kavita Berger noted past work on behavioral threat assessments and asked whether there were methods that could be applied earlier to detect individuals who posed potential biosecurity threats such as, for example, someone stealing an agent or animals, vandalism, violence, or deliberate misuse.

Participants discussed a multilayered approach as raised by Dr. Schoch-Spana, highlighting the need to ensure that such a system includes public engagement and transparency at all levels. Susan Wolf asked about the potential value of having a formal FACA committee for GOF studies of concern. Dr. Schoch-Spana commented that a FACA committee would satisfy one level of engagement and could be beneficial, but one should think of shared governance across all levels. She cited the systems in place at Duke University and St. Jude Children's Research Hospital (see next chapter) as examples worth studying for approaches to providing a diversity of views and participants. She commented on the need for more efforts to collect and share best practices about ways to improve biosafety, biosecurity, and what she called "bio-credibility." The potential additional burden imposed on scientists involved with GOF studies of concern from participating in further public deliberation exercises was raised by Margaret Kosal from the Georgia Institute of Technology. She asked if this was another unfunded mandate to educate a sometimes ignorant public that might be hostile to science for reasons that have not come up in these discussions.

David Drew from the Woodrow Wilson Center introduced himself as a "concerned citizen" who had not been familiar with the GOF controversy before the symposium. He raised the issue of whether the type of public engagement by scientists represented at the symposium was actually a form of "upstream engagement," which can be interpreted as designed to defuse the public's concerns without really addressing them. Dr. Schoch-Spana responded that to be effective the public deliberation process needs to be a shared dialogue that leads to mutually agreeable common ground, not just pure persuasion. Silja Vöneky from the University of Freiburg said she appreciated the stress on the value of ensuring that culture change comes from within scientific disciplines. But she also noted other strong incentives for scientists, such as publication, and suggested broader consideration of opportunities to nudge scientists to strengthen their focus on the safety and security implications of their work. Dr. Huising commented that the issue of culture change is sensitive when one is dealing with elites. In this case one was dealing with highly educated elites who are used to substantial autonomy and are not necessarily very open to ideas that are coming from elsewhere. She believed strongly that the ideas about the importance of safety and security in science are going to have to come from some of the best researchers in each

discipline. "We need the leaders in these disciplines to model the importance of these values and normative expectations in research. We need the journals to expect it and conferences to highlight it. It will have to be pushed from within to be effective."

3

Issues for U.S. Policy

THE POLICY LANDSCAPE IN THE UNITED STATES

Michelle Mello from Stanford University, a member of the Symposium Planning Committee, introduced the theme of the session. Key Findings 2 and 3 of the National Science Advisory Board for Biosecurity (NSABB) Working Paper (NSABB, 2015a: 3-4) addressed the adequacy of the policy frameworks in the United States to provide oversight of gain-of-function (GOF) studies of concern. Finding 2 indicates that the frameworks are effective overall, yet Finding 3 suggests that their adequacy for managing the risks associated with GOF research may vary, depending on which pathogen is being studied.

She commented that there seemed to be plenty to discuss about where the policy frameworks may and may not be adequate or optimal for addressing these risks. To that end, the speakers were asked to reflect, depending on their institutions, on the issues facing federal agencies in administering this regulatory framework as well as some of the strengths and weaknesses in the current policy framework and opportunities for optimizing oversight of this area of research.

Gerald Epstein from the Department of Homeland Security reviewed the scope of the NSABB proposal in terms of who is covered, which pathogens and activities are covered, and what is required. In terms of the existing policy context, he wanted to differentiate between those that are in effect by force of law, and therefore affect all researchers in the United States, as opposed to those that are, for example, a condition of government funding, which would directly affect only the recipients of that

funding. There may be indirect effects in other areas, but a funding hook would only directly affect recipients of U.S. government funding.

The first part of the existing regulatory context that affects everyone in the United States is the laws in place to prohibit biological weapons development.[1] This statute is the mechanism by which the United States implements the Biological and Toxin Weapons Convention, an international treaty which prohibits development or acquisition of biological weapons. Unfortunately, the law does not contain definitions of prohibited types of activity or agents, so he thought the level of subjective judgment involved in proving a violation makes prosecution difficult.

Partly for that reason, the Select Agent Regulations were developed and expanded through a series of statutes.[2] This is a comprehensive set of safety and security requirements governing any use of certain listed pathogens. Of the GOF pathogens, three of them—1918 flu, highly pathogenic avian influenza, and severe acute respiratory syndrome (SARS)—fall under these regulations. The Middle East respiratory syndrome (MERS) does not.

Under the Select Agent Regulations, institutions have to be registered, researchers and staff have to be vetted by the government, and the institution has to have permission to use those agents. There are requirements for safety and security and for incident response and reporting associated with use of these pathogens. And in this case, the government does not have to prove intent. If one is found with one of these agents and has not registered with the government, it is a violation of a law. When that law was passed, it was also recognized that there are legitimate and important reasons why these agents need to be used in research. This is why there is a process by which research institutions and people can become vetted and approved to work with these agents. But it does provide a barrier for people who are not within that scheme.

A third area of legislation that binds everyone in the United States is export controls. These affect the export of certain listed pathogens from the United States or the communication of certain nonpublic, proprietary information that could, for example, include information about how to develop a particular strain of a pathogen if that were not published in the open literature. Information that is published in the course of fundamen-

[1] The primary statute to implement U.S. obligations under the Biological and Toxin Weapons Convention is the Biological Weapons Anti-Terrorism Act of 1989 (Public Law 101-298, May 22, 1990).

[2] The Select Agent program was created by the Antiterrorism and Effective Death Penalty Act of 1996 (Public Law 104-132, April 24, 1996). Following the attacks of September 11, 2001, and anthrax mailings, the program was expanded by the USA PATRIOT Act of 2001 (Public Law 107-56, October 26, 2001) and the Public Health Security and Bioterrorism Preparedness and Response Act, known as the Bioterrorism Act of 2002 (Public Law 107-188, June 12, 2002).

tal research is not affected by export controls, but there is a set of statutes and regulations that could have some bearing on the ability to do and disseminate biological research.

Because they had already been discussed, Dr. Epstein touched only on the policies that are attached to government funding. This includes the federal and institutional policies for oversight of dual use research of concern (DURC), which among the GOF pathogens covers only 1918 flu and H5N1 highly pathogenic avian influenza (White House, 2012, 2015b).

He commented that another framework developed by the Department of Health and Human Services (HHS) for certain H5N1 and H7N9 strains was very similar to the structure of the NSABB's recommendations (HHS, 2013). Lawrence Kerr would describe the framework during his presentation.

Dr. Epstein cited *Biosafety in Microbiological and Biomedical Laboratories* (CDC and NIH, 2007) and *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH, 2013), extensive biosafety and biosecurity guidance that the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) developed for use by anyone doing biological research. It is obligatory as a condition of NIH funding for institutions to follow these processes. This set of best practices enables research using potentially dangerous pathogens to be done safely both for those working in the laboratory and for those in the community. Beyond the formal requirements, this guidance is used widely around the United States, not only for government-funded work, and indeed around the world. Even a policy that nominally has only the force of its ties to government funding can have much greater influence.

Finally, with the caveat that he was not a lawyer, Dr. Epstein cited the issues of the liability that any institution working with potentially hazardous substances could face. Any entity working on something that could pose a risk to its workers, to the neighborhood, or to the environment has to do so in recognition that if there were an accident that causes damage in the community they could be held financially liable. This includes not only harm to the institutions or employees but also harm to the general public. And the extent to which an institution could be held liable may depend on the degree to which there is a regulatory structure in place and whether the institution had been complying with those regulations.

Dr. Epstein commented that any additional development of policy related to GOF research would have to be embedded in the already existing frameworks, and the question of whether these existing procedures would have to be modified to fit the new one or whether they would sit on top would have to be determined as the policy process went ahead.

Dr. Kerr from HHS provided an overview of HHS framework for research with certain highly pathogenic avian influenza viruses (HHS,

2012). Dr. Kerr noted that during the research life cycle, there are points at which biosecurity concerns could be addressed, but it is too difficult—and too damaging to the research enterprise—to do this at the publication stage. Therefore, HHS focused on the research proposal stage as part of the funding award process. He noted that within the existing HHS GOF policy, the focus is on studies that could produce an agent with increased pathogenicity or transmissibility via respiratory droplets. In such cases, an extra level of review is required. The results of the review determine whether a proposal goes on to departmental level review. Seven criteria are taken into account during these reviews (see Box 3-1).

The department-level review provides multidisciplinary expertise—including public health, scientific, security, intelligence, countermeasures, and preparedness and response—from a number of agencies to evaluate these proposals. The department-level review will also identify any additional risk mitigation measures that should be required, and it will determine whether a given proposal is acceptable for HHS funding. For proposals that are deemed acceptable, the funding agency within HHS will make the final funding decision. Dr. Kerr indicated that only a small number of research proposals had undergone a departmental review, but he commented that the results reflected the full spectrum of what one might expect from a review process if it is working well. Some proposals received full approval by the committee and were recommended for funding to the funding agency director. There were also research proposals

BOX 3-1
Criteria Used to Determine If Research Is Relevant to the HHS Framework for Certain H5N1 and H7N9 Influenza GOF Studies

1. Such a virus could be produced through a natural evolutionary process;
2. The research addresses a scientific question with high significance to public health;
3. There are no feasible alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach;
4. Biosafety risks to laboratory workers and the public can be sufficiently mitigated and managed;
5. Biosecurity risks can be sufficiently mitigated and managed;
6. The research information is anticipated to be broadly shared in order to realize its potential benefits to global health; and
7. The research will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research.

SOURCE: Kerr, 2016.

that were received in which individual experiments were rejected by the committee and it was recommended to the funding agency that those not be funded.

Richard Frothingham from Duke University provided an overview of the review process for dual use research instigated by their Institutional Biosafety Committee (IBC), which has been reviewing research for dual-use potential since 2003. Its experiences were recounted in an article in *Science* in 2007 (Davidson et al., 2007). The committee determined that most projects with significant dual use potential were GOF studies, and as a result, it added seven questions to its recombinant DNA registration form in 2005. It also undertook specific training for IBC members on dual use research. The IBC has examined all research including recombinant DNA, select agents, and all research under BSL3 conditions as well as other research upon request.

The Duke IBC does not use a specific dual use definition or threshold but has identified relevant research through the NIH study section or program officer, by the principal investigator's (PI's) answers on the recombinant DNA registration form, or by members of the IBC during the course of its research reviews. Specific examples of GOF identified by the Duke IBC were provided, including cytokine expression by Ectromelia; virulence factors in uropathogenic *E. coli*; adaptation of dengue virus for growth in *Drosophila* cell lines; and HIV infectious molecular clone pseudotyped with vesicular stomatitis virus-G (VSV-G) for initial entry into renal cells. The Duke IBC had learned a number of lessons from having reviewed this research, including:

1. GOF studies were encountered regularly as part of the broad category of dual use research, but the IBC had yet to encounter GOF studies of concern.
2. PIs have had challenges with the concept of dual use research; it was possible to reach consensus on the dual use potential of most biomedical research, but not on specific categories (e.g., DURC).
3. Focusing on specific risk mitigation strategies rather than whether a particular experiment was DURC did enable the IBC to reach consensus, and no GOF research proposals have been rejected.
4. The Duke IBC had received external expert advice on some studies as part of the review process and this had been helpful.

Dr. Frothingham noted that the comparative scarcity of events involving the misuse of research to cause harm makes it difficult to measure the benefits from dual use reviews. He did highlight their value in building public trust in responsible science. He suggested that the early review of GOF research might reduce wasted effort by scientists and improve peer

review and funding outcomes. He concluded by providing a number of perspectives on the NSABB recommendations (see Box 3-2).

Philip Potter from St. Jude Children's Research Hospital introduced the hospital's work on influenza, including its status as one of the National Institute of Allergy and Infectious Diseases' Centers of Excellence for Influenza Research and Surveillance and a World Health Organization Collaborating Center for Studies on the Ecology of Influenza in Animals. Dr. Potter noted that, as a result of their work with influenza viruses, St. Jude is likely to be affected by decisions over the oversight of GOF studies of concern. He highlighted the existence of a specific DURC committee that consists of both scientists and nonscientists. In their system, the PI is responsible for presenting the risks and benefits of the proposed studies. To assist the committee, St. Jude has developed internal guidance on what they should consider. This included ensuring that no GOF virus is resistant to antiviral agents, that suitable vaccines are available, and that advice about the challenges of evaluating risks and benefits in "gray" areas, such as research altering host range and or tropism, is available. The committee has also embraced the ferret as the gold standard for biological testing, requiring its use in all relevant experiments.

The DURC committee has also subjected all experiments involving H7N9 influenza virus to the same scrutiny as the 15 agents covered by DURC requirements. Dr. Potter noted that the DURC committee does

BOX 3-2
Perspectives on the NSABB Recommendations Drawn from the Experiences of the Duke Institutional Biosafety Committee

- GOF is easier to understand than dual use.
- The proposed definition of GOF studies of concern is much clearer than the current DURC definition. It should be possible to reach consensus in determining when GOF research is a GOF study of concern.
- The GOF studies of concern world should be small and definable. There will be substantial overlap with Select Agent programs. Institutional experience with Select Agents will be valuable in implementing GOF review.
- The current IBC or Institutional Review Entity (IRE) mechanisms seem appropriate for institutional GOF review. Institutions should have a low threshold for requesting external expert advice.
- Duke recently moved dual use review, including GOF review, out of the public IBC space to a confidential IRE. The process of GOF review should be transparent but the content is often inappropriate for public disclosure.

SOURCE: Frothingham, 2016.

not publish minutes. He also provided a number of perspectives on the NSABB draft recommendations and broader policy frameworks (see Box 3-3).

Discussion

The discussion that followed addressed local adaptation of the research covered in assessment of DURC or GOF studies of concern. J. Patrick Fitch from the Battelle National Biodefense Institute, a member of the NSABB, speaking in his personal capacity, raised the question of who would be responsible if something went wrong—the scientist or the committee? He also commented that his institutional committee had an experience similar to Duke's. In that situation, a focus on developing appropriate risk mitigation plans for relevant research, rather than on identifying a specific experiment as "DURC," had proved to be a much more productive approach to achieving the same goal.

Following a suggestion by planning committee member Barry Bloom from Harvard University, speaking in his personal capacity, participants also explored whether there was a need for separate IBCs, DURC committees, and possibly GOF studies of concern committees. Some participants felt that it might be possible to combine committees, especially if there was access to the additional expertise that might be required for new roles. Allison Mistry from Gryphon Scientific also proposed updating the DURC requirements to reflect GOF studies of concern and to

BOX 3-3
Perspectives on the NSABB Draft Recommendations and Broader Policy Frameworks

- This is a good initial draft that provides guidance to PIs and Institutional officials.
- The criteria for assessing GOF research are reasonable, but are not specific (terms "highly," "significant," and "likely" should be better defined).
- DURC Committees would likely have the expertise to assess GOF research.
- It is unclear whether local IBC and DURC committees can add additional science as DURC or GOF—if so, this might this lead to a patchwork of institution-dependent rules.
- GOF guidelines need to be crystal clear.
- Need to be specific about who to contact if issues arises.
- If the PI can justify risk/benefit to DURC/IBC committees and the U.S. government, should any GOF studies be prohibited?

SOURCE: Potter, 2016.

avoid creating a separate definition and policy oversight process for GOF research. Christopher Park from the Department of State stressed the importance of the scope of GOF studies of concern in covering both biosafety and biosecurity issues, which set it apart from the DURC process. Richard Frothingham expressed a concern that the DURC institutional process was considerably more cumbersome than the normal IBC process, and he would be reluctant to see them combined.

Dr. Frothingham highlighted the importance of the independence of review committees and their ability to access external expertise. He and Philip Potter discussed the advantages, for example, of including local public health officials in the membership of the IBCs, which both Duke and St. Jude do. Both also have regular contact with the Federal Bureau of Investigation's local Weapons of Mass Destruction coordinator. The importance of clear definitions was stressed by Diane DiEuliis from the National Defense University, while others, such as Mr. Park, highlighted cases where overly detailed definitions undermined the intended aim of the measure. There was a discussion about whether it was better to limit the scope of research likely to be captured under these definitions or, as proposed by Mr. Park, to have a fast-track process for removing research not deemed relevant during the review process. The unique nature of each research proposal was stressed by Dr. DiEuliis, as was the need to consider each proposal in context.

Several participants noted the importance of exploring alternative approaches to GOF studies of concern whenever possible, and the panelists discussed several specific examples of this happening. The value of broader expertise and nonspecialists in identifying alternative research approaches was noted in this regard by Drs. Frothingham and Potter.

Michelle Mello and several of the panelists felt that public trust was an important metric for assessing the efficacy of regimes for DURC and GOF studies of concern. Other participants suggested that reviews of DURC and GOF studies of concern were sensitive and should not be publicly available. Some participants argued that transparency was important and that relevant records should be made available. Both Dr. Frothingham and Gerald Epstein commented on the difficulties posed by the competing goals of protecting potentially sensitive information and ensuring transparency as part of gaining public trust. There were suggestions by Mr. Park, for example, that such information might be made available but not widely distributed.

The discussion also identified a number of tools that might strengthen future efforts. Gregory Koblentz of George Mason University highlighted the importance of learning from past experience. He and other participants called for mechanisms to capture lessons learned in a more systematic fashion. Professor Koblentz also called for additional help for PIs

to understand the underlying concerns that drive assessments of DURC and GOF studies of concern. He also proposed more support to assist regulators in understanding what is possible at the laboratory level and to enable public understanding of the research.

There was also some discussion of the proposal from Silja Vöneky from the University of Freiburg to require laboratories to take out insurance against the risks of GOF studies of concern. Dr. Epstein saw the utility of this proposal—the Department of Homeland Security requires the laboratories it funds for Select Agent research to have insurance. He felt that it would be a useful approach for improving good behavior, although he saw challenges for insurance companies in developing accurate actuarial calculations on these risks.

The definition of GOF studies of concern contained in the NSABB WG's Draft Working Paper was revisited. The third criterion for defining GOF studies of concern was once again the most discussed by some participants, such as planning committee member Dr. Bloom from Harvard University, speaking in his personal capacity, finding no difference between the transmissibility criteria and the one connected to innate or acquired resistance to public health interventions. In connection to the third criterion, Dr. Bloom also raised issues of justice and equity around access to drugs in many parts of the world.

BEST PRACTICES TO INFORM POLICY DESIGN AND IMPLEMENTATION

Philip Dormitzer from the Pfizer Vaccine Research and Development Unit, a member of the Symposium Planning Committee, introduced the session as a continuation of the earlier plenary session on the U.S. policy landscape. This session would present the perspectives of several different key stakeholders, including regulatory agencies and the vaccine industry.

Michael Callahan from the Massachusetts General Hospital and Harvard Medical School opened by highlighting that U.S. efforts to balance the risks and benefits of GOF could be altered for use in other contexts and adapted to the needs of different countries. Dr. Callahan stressed the interconnected nature of the research and development enterprise in the life sciences and for biotechnology, asserting that "the world is flat for bio-innovation." As an example, Dr. Callahan noted that more viral pathogens have been sequenced in China in 4 months than have ever been sequenced in the United States and Europe.

A major theme of Dr. Callahan's remarks was that market-driven and beneficent GOF research is already happening around the world, all of which is outside of the U.S. and European policy and regulatory frameworks. One of his main points was that U.S. and European vaccine

production does not always take cultural and other factors into account. For example, he noted that Western vaccines will only be used in Indonesia if they conform to requirements that make them halal. Another relevant example discussed was the production in Asia of effective and inexpensive H5N1 poultry vaccines. The life span of a chicken in Asia is about 6 months, so Western vaccines costing $7 per dose are not going to be used when Asian-produced vaccines costing pennies per dose are available. He also noted that countries in the group of 112 Non-Aligned Nations may refuse to share pathogen gene sequences with U.S. scientists because "they've been ripped off." He suggested that the U.S. government needs to protect "our international collaborators from R01-funded investigators who seek to do nothing more than get a virus, go home, and write their big *Nature* paper." Dr. Callahan concluded with a series of recommendations for aligning domestic and foreign policies relevant to GOF (see Box 3-4).

Robert Fisher from the Food and Drug Administration (FDA) introduced the FDA's main mission: to ensure that medical products and associated technologies are safe and effective. Dr. Fisher discussed a variety of regulations relevant to the evaluation of products and the implementation of regulatory mechanisms. He highlighted a number of approaches used by the FDA, including randomized clinical trials, surrogate endpoints, and animal efficacy data. He stressed that, regardless of the approach taken, the FDA relies on data for its decision making.

Dr. Fisher noted that the GOF framework focuses on specific agents of concern, or particular pathways of concern, potentially impacting

BOX 3-4
Recommendations for Aligning Domestic and Foreign Policies Relevant to Gain-of-Function Research

- Introducing Institutional Review Boards and Biological Weapons Convention guidance to foreign venture capitalists, incentivizing market entry through compliance.
- Licensing safe, effective, and inexpensive vaccines to foreign markets or exporting rational vaccine designs to foreign providers.
- Ensuring U.S. government–qualified expert review of academic claims for biosafety and pathogen research in foreign research facilities.
- Incentivizing host nation compliance through the use of metrics which demonstrate local benefit.

SOURCE: Callahan, 2016.

FDA-relevant research. However, the narrowing of focus to GOF studies of concern reduces the potential impact considerably. He provided examples of where GOF studies of concern might impact the work of the FDA, including the production of vaccine seeds from molecular clones, or adapting vaccine candidates to grow in cell-based systems rather than egg-based systems. He noted the potential for GOF concerns to impede rapid, large-scale production of vaccines to meet seasonal and emergency needs.

Lessons from the FDA's regulatory experience were also provided: for example, the importance of early and sustained engagement with stakeholders. Dr. Fisher stressed the value of ensuring sufficient flexibility in regulatory regimes and for their implementation.

Jonathan Moreno from the University of Pennsylvania framed his comments as both a bioethicist and a patient—or a "consumer" of the public health benefits of GOF research. Dr. Moreno identified a number of questions that he thought needed to be addressed when considering policy options for dealing with GOF research, including the impact of terminology and the response to the term GOF; the implications of mutants—both natural mutations and escape mutants; the potential for a generalized GOF policy being too broad to implement effectively; the adequacy of safety records for quantifying the risks of laboratory accidents; current levels of accident reporting; the need to address basic research and vaccine development activities where more acceptable or safer alternatives do not exist; determining the realities of the relationship between GOF methods and vaccine development; and the role of basic science during a health emergency.

Dr. Moreno identified five areas where he thought there was consensus relating to the controversy over research and the policy options to address it:

- Much regulation fails to hit the mark for this field and could needlessly delay vaccine development
- Some regulation is needed, for both biosafety and biosecurity
- Biocontainment does not have a perfect record
- Risk mitigation often only requires some imagination
- Sometimes there are acceptable alternatives to GOF studies of concern, even if they are not the best option

He also thought there might be agreement that GOF data alone cannot predict emergence of a pandemic (genotypes to phenotypes), but perhaps this is getting better; the long-term potential of pre-pandemic strain selection could be "transformative" in new vaccine development; humans are vulnerable to certain natural strains that could be targeted for research,

such as bat SARS-like coronavirus strains; and animal model development for SARS and MERS should be permitted.

He highlighted opportunities for public deliberation as to whether all three of the characteristics for GOF studies of concern, as proposed by the NSABB, are needed for an experiment to warrant additional oversight, or whether the production of a pathogen anticipated to possess two of the characteristics would be sufficient. Dr. Moreno highlighted the need to build on best practices when developing capacities to review GOF studies of concern. He reviewed the composition and mission of the Wisconsin Bioterrorism Task Force and the Stanford University benchside ethics consultations as examples to be considered. Dr. Moreno presented a potential model for institutional bodies for the operational review of GOF studies of concern: Risk–Benefit Assessment Teams, or R-BATs (see Box 3-5).

Ethan Settembre from Seqirus provided an overview of the global influenza system that addresses variability in influenza viruses to develop and deliver candidate vaccine viruses. Dr. Settembre described an example of how the system works in practice, detailing vaccine generation over a 4-5 month period in response to the H1N1 influenza pandemic in 2009. He noted that while vaccines can be produced increasingly quickly, pandemics

BOX 3-5
A Proposal for Risk–Benefit Assessment Teams (R-BATs)

Risk–Benefit Assessment Teams should work in an informative and consultative (but not dispositive) manner. The team should encourage researchers to demonstrate that they have considered the risks and benefits at the current stage of their work. These teams are intended to move beyond a paper mechanism to a dynamic, real-time process. In particular, they should

- Be independent and multi-disciplinary.
- Represent the perspectives of both science and security communities.
- Work through an iterative process that spans the life cycle of the research.
- Use a schedule based on milestones and perhaps be able to make unannounced audits.
- Make assessments based on case-specific risk–benefit parameters.
- Help to develop and disseminate best practices for research with GOF studies of concern.

Further consideration would be necessary to determine whether their existence should be voluntary or mandatory.

SOURCE: Moreno, 2016.

emerge even more rapidly, necessitating further research and development. Dr. Settembre highlighted a need to further speed up production of vaccines in response to both pandemic and seasonal influenza events. He discussed a synthetic process for generating vaccine candidates using attenuated backbones and available hemagglutinin and neuraminidase sequences that was developed with the J. Craig Venter Institute and Synthetic Genomics Vaccines, Inc.. The process allows the generation of synthetic influenza viruses that are attenuated, but would allow for speed, accuracy, and high yield. He noted that this is one of the ways to make vaccine viruses to address immediate important medical needs in a short period of time to get ahead of the wave of infection. This approach had been used to produce an H7N9 vaccine candidate in 2013.

Discussion

The discussion that followed included both an interchange among the panellists and questions and comments from the audience. Among other topics, the discussion returned to considering different ways of defining GOF studies of concern. Philip Dormitzer pointed out that not all GOF research involves GOF studies of concern, and therefore not all the research needs to be overseen by any additional policy frameworks. Following his comments, panelists and participants discussed the subjective nature of determining what is (and what is not) of concern. Michael Callahan argued that any definition for GOF studies of concern needs to be general, adaptive, and culturally appropriate for foreign scientific communities. Participants noted that specialist terminology might not translate well into other languages and settings: for example, Dr. Callahan noted long-standing issues around the meaning of "biosecurity."

Participants also reexamined the importance of ensuring that the process to consider policy options, as well as any new frameworks it produces, cover both the public and the private sector. The importance of adequate containment for GOF studies of concern was another reoccurring theme during the discussions. The issue of enforcement was also revisited. Possible unintended consequences for new policy frameworks to oversee GOF studies of concern were discussed by Dr. Callahan, and possibilities of a negative impact on vaccine production were considered. The risks of regulatory uncertainty were also addressed, with some participants arguing that regulatory burdens are more acceptable when the "what" and the "why" are clear. Issues around the harmonization of domestic oversight regimes, such as those for DURC and GOF studies of concern, were also highlighted by Robert Fisher. Some participants called for the development of a more overarching framework to deal with risks and benefits from life sciences research.

The international implications of determining thresholds of concern or acceptable risk were considered, as well as international perceptions about why the United States might be concerned about this research. Dr. Callahan suggested that it was important to understand the nature and motivation of relevant international stakeholders to improve the dialogue on GOF research. To this end, he noted the importance of strengthening research collaborations, in particular working more closely with partners inside their countries. Gregory Koblentz asked whether it was time to move beyond stovepiped concepts of "biosafety" and "biosecurity" to adopt a more holistic concept of "biorisk management." Dr. Fisher responded that, from a regulator's viewpoint, to the extent that such an approach could reduce uncertainty, it could be helpful.

There was an exploration of the impact of over-regulating GOF research for countermeasure development. Dr. Dormitzer pointed out that, because one of the factors for identifying GOF studies of concern is the absence of effective countermeasures, limiting research that could provide such measures could be counterproductive. There was also consideration of the opportunity costs of not doing research, especially in justifying potential barriers to developing countermeasures. Issues around intellectual property were also explored, with Dr. Callahan discussing barriers for the development of countermeasures, or barriers to the conduct of science internationally. There was a call to change the incentives for countermeasure development—to produce more players, more stakeholders, and therefore more solutions. To this end, Dr. Callahan recommended that greater attention be paid to foreign industry, as an increasing number of products and self-sufficient markets were being developed.

Challenges in disease surveillance were discussed. While some participants suggested that knowledge produced by GOF research could be useful for detecting emerging pathogens, others noted the lack of current surveillance capacity. Current shortcomings in data sharing and capacity for disease surveillance can also distort risks from disease and the impact of public health measures, according to Dr. Callahan. He also noted international concerns that disease surveillance data and capabilities are being used for nonpublic health purposes.

4

International Policy

INTERNATIONAL DIMENSIONS OF GAIN-OF-FUNCTION RESEARCH

Barry Bloom from Harvard University, a member of the Symposium Planning Committee, introduced the session by noting that, as had already become clear in earlier discussions, the issues related to gain-of-function (GOF) research were not confined to the United States. This session would provide background and insights about the international dimensions of GOF research and illustrate the ways in which various organizations outside the United States have been contributing to the discussions from the beginning.

Ruxandra Draghia-Akli from the Health Research Directorate of the European Commission introduced the European Union (EU) innovation framework Horizon 2020. With a budget of €79 billion, the program is intended to support research and development that is increasingly complex, interdisciplinary, and costly, and that also requires a critical mass. It provides a vehicle for increased collaboration across the 28 countries of Europe. The Horizon 2020 framework covers a broad range of research and development activities; most relevant to GOF research is the section on Societal Challenge 1: Health, Demographic Change and Wellbeing, with a budget of €7.4 billion. The research funded under this framework has to have civil or public health applications. Any dual use potential is unintentional.

There has been no specific call for proposals on GOF research according to Dr. Draghia-Akli, but under the health calls, proposals were encour-

aged to strengthen research on prediction, identification, modeling, and surveillance of newly emerging infectious diseases in humans, and to identify factors promoting the emergence of pathogens with human pandemic potential from pathogens with a zoonotic background and related prevention strategies. Both of these areas could potentially result in proposals involving GOF research. Five EU-funded research projects with GOF elements were identified: (i) EMPERIE (European Management Platform for Emerging and Re-emerging Infectious Disease Entities, 2009-2014); (ii) PREDEMICS (Preparedness, Prediction and Prevention of Emerging Zoonotic Viruses with Pandemic Potential using Multidisciplinary Approaches, 2011-2016); (iii) ANTIGONE (Anticipating the Global Onset of Novel Epidemics, 2011-2016); (iv) AntiBotABE (Neutralizing antibodies against botulinum toxins A,B,E, 2010-2015); and (v) TIRAMISU (Humanitarian Demining Toolbox, 2012-2016).

Dr. Draghia-Akli then outlined the ethics review processes that research undergoes in Horizon 2020.

- During proposal preparation, applicants are asked if their proposal has an exclusive civilian focus on research and if their research uses or produces goods or information that will require export licenses in accordance with legislation on dual use items.
- Ethical screening is carried out for each successful proposal by at least two ethics experts, drawing on a variety of different backgrounds, including law, philosophy, medicine, and biology.
- A full ethical assessment for all proposals containing potential dual use issues is carried out by at least five ethical experts.
- At the end of the whole process, the ethics report determines if the project has clearance, requiring no further action; conditional clearance, requiring changes to be made to the description of work (such as requirements for permits, follow-up, or ethical audits); or no clearance, meaning that the project will not be funded.

There has also been specific dialogue with stakeholders in the European Union on GOF research, including the European Society for Virology, which has a common policy for scientific research and publications; the Foundation for Vaccine Research, which has called for a comprehensive risk–benefit assessment of GOF studies of concern; and the European Academies Science Advisory Council (EASAC), which established a working group in autumn 2014 to explore consensus on key questions, identify further GOF issues, and clarify options for policy development. The European Union acknowledged the need to improve awareness and best practices among members of the scientific community and to promote an underlying culture of responsibility, given the potential for

accidental release and misuse. The European Union also welcomed the EASAC working group recommendations (EASAC, 2015a; see the comments by Volker ter Meulen).

Dr. Draghia-Akli provided three different approaches toward implementation within the European Union: researcher-based approaches, as used in the United Kingdom; researchers being overseen by institutions, as used in the Netherlands; and supervision by a national agency, as used in France.

Volker ter Meulen, the chair of the EASAC working group, introduced his institution. EASAC was formed in 2001 to enable European national academies of science to collaborate in giving advice to EU policy makers (e.g., the European Commission and Parliament). Its membership comprises all EU national academies of science plus Norway and Switzerland, and its objective is to deliver consensus outputs to provide a means for the collective voice of European science to be heard. Dr. ter Meulen explained that the EU scientific community had expressed differing views to the president of the European Commission in 2013 on relative benefits and risks of GOF influenza virus (H5N1) research (European Society for Virology, 2013; Foundation for Vaccine Research, 2013). As a result, the European Commission and its chief scientific adviser requested EASAC to clarify and advise on these issues. EASAC brought together scientists, nominated by its member academies, who represented a mix of expertise and a wide range of views about the GOF controversy. The group sought to find areas of consensus as well as issues that had not been resolved. Its report also offered recommendations about what further analysis would be necessary to assess future options for research with potentially pandemic pathogens. The report also identified which of the European Union's current regulations applied to GOF research, how national- and EU-level responsibility should be divided, and what best practices already exist at the national level that could inform other countries.

During the course of this work, EASAC identified a range of critical issues to consider when addressing GOF research as well as key messages, which are summarized in Box 4-1.

EASAC has subsequently produced messages to academies of science worldwide, policy makers in EU institutions, and EU member states as well as to research funding bodies, regulatory bodies, professional societies, and others in the scientific community. It has also worked to catalyze further broad engagement via member academies.

Dr. ter Meulen concluded by providing some insights for strengthening international consideration of GOF issues, including the importance of addressing differences in understanding and in systems between countries and regions; ensuring layered, integrated approaches; building

BOX 4-1
Key Messages from the European Academies Science Advisory Council's Work on Gain-of-Function Research

Self-regulation and harmonization

- Good practice requires conforming with regulations, safety provisions, codes of conduct, and justifying proposed research.
- Self-regulation means instigating a series of checks and balances on research within the scientific community. It requires raising awareness among researchers and their institutions, thereby necessitating education.
- Attention to biosafety issues is needed at all stages of the research life cycle.
- There is a continuing role for Academies of Science in promoting biosafety and biosecurity norms and supporting audits of research practices.

Benefit–risk assessment

- This is not a "once and for all" calculation but a continuing, collective commitment to understand and communicate the issues.
- Incommensurable parameters measured in risk and benefit do not allow a value-free determination to be made.
- Questions remain as to the feasibility of quantifying benefit as prospective public health impact or describing its impact on the generation of scientific knowledge.
- Academies and learned societies need to continue to promote discussion across the scientific community and with other stakeholders.

EU/national activities and organizations in biosafety and biosecurity

- There is a possible role for the European Commission (DG Sante) Health Security Committee in collating available information.
- Guidance is needed for research funded by Horizon 2020 as well as at the national level.
- All researchers and institutions need to conform with EU regulations as implemented nationally.
- No new EU-level body was recommended.
- Member states should have clear national advisory approaches and governance mechanisms with statutory powers.

Publication of sensitive information

- Researchers and their institutions all have responsibility to make decisions about publishing sensitive information.
- Journals should be encouraged to seek appropriate advice, including from security experts.
- Export control regulations are an inappropriate and ineffective vehicle to block publication.
- The European Commission's (DG Research) attempts to raise awareness about revision of these regulations are welcome—researchers should continue to inform policy-makers about these issues.

continued

BOX 4-1 Continued

Public engagement

- Trust and openness are crucial for researchers and their institutions.
- Academies and others in the scientific community should actively participate in public dialogue—articulating objectives for research, the potential for benefit and risk, and biorisk management practices adopted.
- EASAC is committed to continuing working with academies to promote engagement.

SOURCES: EASAC, 2015a,b; ter Meulen, 2016.

links between researchers, policy makers, and other stakeholders; and a continuing commitment to public engagement.

Silja Vöneky from the University of Freiburg discussed the German Ethics Council (GEC) report on biosecurity from 2014 (German Ethics Council, 2014). Dr. Vöneky introduced the GEC, noting that it is an interdisciplinary independent counsel of experts whose 26 members are appointed by the president of the German parliament. She reviewed recent work exploring options for biosecurity for research and health and noted that the GEC report explored biosecurity issues but not biosafety because the regulatory regimes associated with biosafety are much more developed.

Dr. Vöneky presented five recommendations for future GOF research based on the findings of the GEC report (see Box 4-2). These recommendations reflected those proposed for use within Europe, focused on five different areas of governance: raising the level of awareness on biosecurity among the scientific community; elaborating national biosecurity codes of conduct; reviewing research funding; making specific national recommendations tailored to national needs; and developing European and international initiatives.

In the German context, Dr. Vöneky recalled that the GEC had recommended that an appropriate definition of the research of concern should be included in an act of parliament; the definition should be further developed by detailing relevant groups of experiments in a statutory instrument or regulation; and a list of agents associated with the research should be developed. She commented that the list of agents will need to be updated to reflect advances in the life sciences, suggesting that it should not be listed in legislation.

Dr. Vöneky noted that it was difficult to assess the impact of these recommendations over the year since they were made. She highlighted

BOX 4-2
Recommendations for Future GOF Research Based on the Findings of the GEC Report on Biosecurity

Raising awareness in the scientific community—To promote responsible research and improve knowledge of, and access to, relevant resources. One approach to raising awareness was to integrate biosecurity components into undergraduate and graduate life science curriculum.

The use of codes of conduct—Codes were deemed to be practical tools to define responsible approaches for dealing with biosecurity challenges, including by detailing concrete obligations to minimize risk. They were felt to be useful instruments for self-regulation and can be supplemented by broader standards. Codes of conduct could be adapted to address GOF issues, including thresholds for GOF studies of concern.

Strengthening the role of research funding in ensuring responsible conduct—Funding of GOF research should require adoption and adherence to the above code of conduct. Specific funding guidance should be developed ensuring that GOF studies of concern are not funded when there is no need to use GOF approaches or when the risks outweigh the benefits.

Establish a new commission to oversee GOF studies of concern – An independent body, with interdisciplinary membership and participation by civil society, should define GOF studies of concern, conduct risk–benefit analysis of specific research proposals, decide on any additional measures to mitigate or manage risks associated with the research, and undertake relevant consultative roles. It should become a legal obligation to consult the commission before undertaking GOF studies of concern.

Regional and international engagement—Common standards can play an important role in addressing biosafety and biosecurity concerns related to GOF studies of concern. Efforts within scientific communities should continue to develop a common understanding on what constitutes responsible research. An attempt should be made to develop an international code of conduct. At a regional level, States should advocate for a common position on the funding of GOF studies of concern. States should also work internationally to define and classify dual use research of concern (DURC) and GOF studies of concern and appropriate biosafety and biosecurity precautions for undertaking such work. A new international instrument to define the fundamental principles and limitations of GOF studies of concern should be negotiated. This could be a formal treaty or more likely a soft law instrument.

SOURCE: Vöneky, 2016.

progress in Germany in the promulgation of codes of conduct to address DURC issues, and she discussed the implementation of such codes in a number of research institutions.

Dr. Vöneky also highlighted a number of other results that might be relevant to GOF discussions. She believed that soft measures, such as requirements connected to funding, might be less suitable in the EU context. She suggested that measures to evaluate and manage risks associated with GOF research would need to be either codified by states into appropriate laws and regulations or contained with other legal frameworks such as constitutions or international treaties because of competing interests between the rights and freedoms of science and scientists and rights associated with the right to life and health for other parts of the population. Dr. Vöneky noted that the work undertaken by the GEC revealed that existing legal rules governing GOF research in Germany and Europe are insufficient and need to be more coherent. She highlighted internal inconsistencies around the publication of results and funding arrangements.

Dr. Vöneky concluded by stressing the need to balance scientific freedoms and responsible research with the need for proportional measures that do not unnecessarily impede research but do help to manage risks. She reflected on thresholds for GOF studies of concern, suggesting that such a concept might usefully capture experiments that might result in pathogens, which increase the danger of an epidemic of a severe human disease. She felt that such experiments should not be undertaken unless a direct, concrete, and overwhelming benefit for life or human health is probable.

Keiji Fukuda from the World Health Organization (WHO) explained that the world is currently facing a broad mix of issues and uncertainties related to genetic technologies and their potential to do harm, such as GOF research. Other approaches, such as synthetic biology, offer ways to generate novel organisms. Furthermore, Dr. Fukuda noted that the nature of these challenges is evolving as access to the necessary technologies changes: for example, through the emergence of cheaper technology and the advent of private community laboratories. Dr. Fukuda also noted that the entry into force of the Nagoya Protocol on sharing the benefits of biological resources also impacts this space. Furthermore, he recalled that developments, such as the WHO Pandemic Influenza Preparedness Framework deal with not only the movement of viruses but also the movement of sequence information. Dr. Fukuda noted that while these are critical international issues, global awareness of them and how they intersect remains minimal. He highlighted the lack of a clear strategy and outstanding questions as to whether they should they be dealt with separately or were better addressed together. Dr. Fukuda recalled that while

risk assessment can be a scientific and precise process, risk perception, tolerance and management are cultural, political, and, at the global level, consensus based. Dr. Fukuda presented four options for further work on GOF research (see Box 4-3).

Discussion

Barry Bloom led a moderated discussion among the panelists. They explored opportunities for interaction between U.S. and European efforts to address GOF research, with Dr. Draghia-Akli expressing how beneficial such exchanges on common policy problems could be. The importance of information exchange was repeatedly expressed. One panelist felt that the European Union was likely to be flexible about approaches to the biosecurity aspects of GOF research, but noted that given the highly developed arrangements already in place there may be less opportunity to influence biosafety policy. The complexity of the European regulatory architecture was also noted, with one panelist suggesting that additional measures were added but rarely replaced existing arrangements. A trend toward the European Union engaging international partners was highlighted, especially through the development of principle-based voluntary frameworks that could be implemented by partners. Past collaborations between the United States and the European Union were noted on health and biomedical related policy development: for example, bringing together funding agencies to streamline work on rare diseases. Past examples also included collaboration on sensitive issues, such as data and sample sharing.

BOX 4-3
Four Options for Further Work on GOF Research

- Options for going forward will necessitate developing global consensus on technical aspects, such as issues, principles, definitions, and terminology.
- At an operational level, there are examples of programmatic activities implemented by WHO that might offer models, such as the prequalification of laboratories, the oversight of smallpox research and the inspection of the laboratories conducting research.
- Multilateral forums such as the Biological and Toxin Weapons Convention or the International Health Regulations might be suitable venues for addressing specific aspects of GOF but will likely be time consuming.
- Member state funding and support is essential regardless of what approach is taken.

SOURCE: Fukuda, 2016

Panelists also considered options for attempting to ensure that GOF studies of concern were conducted only under appropriate safety conditions. At the suggestion of Barry Bloom, panel members discussed precedents used elsewhere for the prequalification of appropriate laboratories, assessing them against predetermined capabilities: for example, those used for quality control of laboratories used by United Nations agencies. Several participants supported such an approach but highlighted that it would be necessary to consider carefully what the desirable capabilities would be. Other participants felt that the GOF studies of concern context was considerably more complicated than the purposes for which prequalification has been used in the past, and they suggested that the desirable capabilities would be too context dependent for such an approach. They also noted that the number of relevant facilities might be larger than those found in other areas where prequalification has been used.

An open discussion followed and consideration of prequalification of laboratories continued. Gavin Huntley-Fenner questioned which international organization might oversee such an approach. WHO, the International Standards Organization, and the United Nations Educational, Scientific and Cultural Organization were discussed. Keiji Fukuda stressed the importance of any hosting organization being perceived to be neutral and having the trust of key stakeholders.

Issues around standards and harmonized approaches were also explored. Harvey Fineberg, chair of the Symposium Planning Committee, noted that in certain cases—such as for the approval of medicines, drugs, and other medical devices—there was still a notable degree of difference in what is approved, and when, despite a comparatively common agreement on the characteristics to be assessed, relatively straightforward measurements, and well-established decision-making processes. Other participants noted that in the European context, while risk assessment might be carried out collectively, regulatory approval still happened at the national level. Some participants suggested that the chances of creating a common system for GOF studies of concern in the short term were small, especially given the absence of a common definition. Other participants noted that the number of scientists and laboratories potentially conducting GOF studies of concern was currently limited and that there might be opportunities to develop common standards— for example, for biosafety precautions—among the relevant community.

The possibility for developing common approaches between the United States and Europe was also explored, with Silja Vöneky suggesting that reaching such an agreement might help jump-start a broader international process. Michael Callahan felt that a broader buy-in from the start would help legitimize the process. To underscore that argument, Piers Millett from Biosecure transmitted the views on GOF of the 112 states that

comprise the Group of Non-Aligned Movement and Other States under the Biological Weapons Convention (BWC) by reading aloud from the Group's statement to the BWC.[1]

There was also an exploration of whether harmonization efforts should be scientist-led or state-led, with different participants favoring different models. Some participants noted that, at present, GOF studies of concern were largely confined to public institutions, enabling governments to play a leading role. Others noted that only a limited number of states had so far shown an interest in GOF studies of concern, suggesting that a scientist-based, bottom-up approach may help increase government interest around the world. There were also discussions of whether a formal approach was needed, requiring international instruments, or a more informal approach might be more suitable, perhaps through appropriate guidelines such as those used to underpin international efforts on infection prevention and control. Participants also discussed the value of strengthening a culture of responsible research among relevant scientific communities, noting that they had key insights into the risks associated with GOF studies of concern.

The changing distribution of research capacities, sources of funding, and the markets they serve were also discussed. Some participants noted that these developments complicated efforts to address GOF studies of concern, and others noted that it required additional efforts to understand a broader variety of motivations for and contexts within which GOF research might be conducted. Participants discussed incentivizing industry participation, alternative funding strategies and business models in general, and public–private partnerships in particular, for dealing with changes in markets, funding and demographics. Participants provided a number of examples of successful precedents, including the Innovative

[1] The statement is "there have been recent advances demonstrating the increasing sophistication of synthetic biology, together with other enabling technologies, which have benefits, together with the potential for uses contrary to the provisions of the Convention. All states must conduct such activities in a transparent manner, in order to build the confidence of other States Parties. There is a need to regulate these activities, to ensure that they do not lead to any concerns related to ethics, safety and security as well as any uses contrary to the Convention. This has assumed added importance in the light of reports concerning experiments that have been taking place on highly contagious virulent flu strains like H5N1, as well as the production of several new strains of viruses that are both contagious and deadlier than the 1918 Spanish flu that killed almost 50 million people, and the discovery of the deadly smallpox variola virus dating back to the 1950s. Such regulation must, however, be undertaken in a manner that does not hamper scientific and technological developments that are in keeping with the spirit and letter of the Convention, which are of benefit, more especially to developing countries." It is available at http://www.unog.ch/80256EDD006B8954/(httpAssets)/DF2D9E3CAA6D5FEDC1257EA400369E6E/$file/NAM+Statement+on+S&T+MX+2015-3+final.pdf.

Medicine Initiative in the European Union, and global networks for building preparedness for emerging epidemics.

OPPORTUNITIES TO HARMONIZE GOF RESEARCH POLICY AND PRACTICE

Ronald Atlas from the University of Louisville, a member of the Symposium Planning Committee, introduced the session. The plenary on the first day provided participants with an awareness of the international context within which the GOF controversy has evolved. The purpose of this session was to look ahead, to explore the potential for increasing international coordination of policy and practice for GOF studies of concern. What are the opportunities in different regions, including those where the research is performed and those where the pathogens of concern are endemic? What roles might national governments take in fostering efforts at coordination? What are some of the international venues, such as regional or international organizations, where discussions could take place and policy options could be developed? What are the roles for national and international scientific organizations?

George Gao from the Chinese Academy of Sciences and the Chinese Center for Disease Control and Prevention began by discussing risk and benefit. Dr. Gao discussed an example of the H7N9 influenza virus, noting that study of the virus could be directed at finding a mutation in the receptor binding site that might be responsible for allowing the virus to switch from an avian to a human host and allow the virus to transmit to humans. Dr. Gao noted, however, that checking all possible genetic combinations is infeasible, and, given the importance placed on finding the mutations responsible, a GOF approach proved most efficient.

Dr. Gao stressed the importance of international collaboration, cooperation, and harmonization. He recalled one case where two researchers, one in the United States and another in China, were collaborating on research connected to Golden Rice. The contents of the underpinning agreement were different in Chinese and English, which led to misunderstandings and substantial impediments to the research. He felt that harmonization was needed on more than just policy development and that it was necessary to have oversight of the research being undertaken. He suggested that it is important to monitor what is happening in laboratories. Dr. Gao noted the need for a suitable international forum for discussions at the government level. He felt that it was important for top officials in many countries to engage with this issue. He suggested, however, that in many countries there were still opportunities for greater domestic harmonization of relevant rules and regulatory approaches.

The interests of scientists often drive the direction and approaches to research, Dr. Gao noted. Therefore, he felt that it was important to engage with individual researchers on these issues. He believed that GOF experiments should be done in highly regulated laboratories and undertaken only by the best scientists.

Gabriel Leung from The University of Hong Kong began by considering GOF research in context. He noted that this was a discussion of risk to humans and, to a lesser extent, ecological security. He underscored that this was an international issue because pathogens do not respect borders. Dr. Leung suggested this is also a global security issue, not only a U.S. national health security concern. He felt that the primary outcome of policy discussions should be conclusions as how best to keep the global population safe from potential consequences of pathogens that were highly virulent, highly transmissible, and/or resistant to public health interventions.

Hazard analysis, according to Dr. Leung, was a critical control point. He highlighted lessons that might be learned from food safety experiences. Dr. Leung suggested that it was necessary to look for the weakest link in the global supply chain and argued that, in the case of GOF studies of concern, it was a lack of public health preparedness. He recalled that the majority of countries around the world had self-declared their inability to meet core requirements under the International Health Regulations. He suggested that investing in global capacity to respond to disease minimizes the proportion of GOF studies of concern that would then be of concern. To this end he commended the recommendations of the recently released report of an international commission hosted by the National Academy of Medicine on which he had served (Commission on a Global Health Risk Framework for the Future, 2015).

Dr. Leung noted that a highly organized regime for governance of GOF research was important for obtaining human security. He felt that the issue had been largely ignored elsewhere in the world. While he acknowledged that a national policy in the United States would have a global impact, he noted that its relevance should not be overestimated. Stringent arrangements in the United States, or a ban on GOF studies of concern, according to Dr. Leung, would not stop risks to the global population from research carried out in other countries.

Dr. Leung also argued that overly burdensome regulations can lead to unanticipated consequences—perhaps driving GOF research underground or possibly relocating it to other countries without such regulations. He expressed concern over how the broad findings of the National Science Advisory Board for Biosecurity might be translated into guidance and implemented by IBCs in institutions. He felt that greater clarity, especially as to what is (and what would not be) permitted, was needed,

as was more guidance on the implementation of the proposed policy framework.

Dr. Leung provided a number of specific reactions to the inputs to the symposium (see Box 4-4) and concluded that responsible science with robust oversight of GOF studies of concern is warranted, but it should "not squeeze the lifeblood out of scientific enterprise." He felt that the balance between the two must be clearly defined and continually fine-tuned.

Nisreen AL-Hmoud from the Royal Scientific Society of Jordan noted that understanding life processes is becoming ever more important in terms of health, nutrition, and industrial application. Dr. AL-Hmoud suggested that the Middle East and North Africa (MENA) region lags behind other parts of the world in addressing issues in life sciences research.

BOX 4-4
Issues for Further Consideration When Considering Opportunities for International Harmonization of Approaches to GOF Research

- Is there any unique value or value-added from GOF studies of concern or can alternative methods exhaustively derive the same knowledge set?
- GOF research has led to new characterization of pathogens as well as follow-up research identifying new markers of mammalian adaptation.
- Is the proposed third criterion of a GOF study of concern truly orthogonal to the other two dimensions of transmissibility and virulence? Is there an advantage, for example, in better defining or making more encompassing the two truly orthogonal axes of transmissibility and virulence as the product of host-agent interactions as opposed to just innate properties of the agent alone?
- Do we need another layer of regulations or could existing regimes be adapted?
- More consideration may be warranted as to the unintended or intended consequences of policy options. Particular focus should be placed on avoiding such heavy burdens on GOF studies of concern that the research is avoided altogether.
- Financing has always been a powerful modifier of behavior and offers opportunities for shaping engagement on GOF studies of concern.
- Conflicts of interest should be avoided. It is to be hoped that situating the bodies reviewing research for GOF studies of concern inside the same agency that funds the research does not provide a conflict of interest.
- The RBA (risk and benefit assessment) is far from a trivial exercise but more work is necessary to definitively resolve the original questions posed. While it is difficult to draw direct lessons from an RBA exercise, we are now in a better place to understand what we do not know and how much we need to learn.

SOURCE: Leung, 2016.

She stressed the importance for the region of greater progress in ensuring that natural diseases are contained as soon as possible; that harmful consequences of research are minimized; and that laboratories operate safely—both for their workforces and for the communities in which they are situated.

While controversies around research involving highly pathogenic avian influenza virus and the Middle East respiratory syndrome coronavirus (MERS-CoV) have generated considerable discussion and debate among virologists, public health scientists, and experts in the United States and certain other parts of the world, a considerable need for raising awareness about GOF research persists in the MENA. This is needed for laboratory directors and policy makers as well as for life scientists. For maximum benefit, Dr. AL-Hmoud argued that policies and practices aimed at reducing and managing risks should be planned in a holistic manner as part of national safety and security strategies. She noted that, while some countries have begun to develop such plans, many others have not.

Dr. AL-Hmoud noted that while risks vary from region to region, and from one country to another, without a common methodology for assessing risks and for having appropriate policies and practices to manage and mitigate these risks, any international effort will be neither comprehensive nor effective. The countries in the region have explicitly recognized the need for comprehensive scientific strategies, and Dr. AL-Hmoud reviewed efforts under the Biosafety and Biosecurity International Conference series as an example.

Dr. AL-Hmoud stressed the public health impact of coronaviruses, in particular MERS-CoV, and she recalled that the antigenic relationships among the different coronaviruses or how these relationships influence the capacity of different strains to emerge in human populations remains uncharacterized. While she noted progress in relevant tools and information, Dr. AL-Hmoud also stressed that important research questions need to be explored further and they must address potential issues around security and select agent status. She highlighted opportunities for the MENA region to learn from the experiences of other regions, to adopt best practices, and to develop networks of experts.

Dr. AL-Hmoud discussed the importance of developing systematic programs that strengthen human capacity for safe and secure handling, importing, and exporting pathogens to strengthen the oversight of GOF research. She highlighted the need for certain infrastructure and policies at the national level. Dr. AL-Hmoud suggested that these programs should offer considerable regional and international benefits by reducing risks from pandemics and epidemics, regardless of whether they are natural, accidental, or deliberate. To reduce the risk of biological accidents, Dr. AL-Hmoud called for better safety standards and practices,

and improved designs and procedures for security systems at biological facilities. In relation to GOF research, Dr. AL-Hmoud also noted the need for better education and training, more awareness raising, detailed consideration of unintended consequences, and broader adoption of best practices and codes of ethics.

Michael Selgelid from Monash University in Australia suggested that too much of the deliberative process and decision making on GOF research had been restricted to scientists. He argued that there had not been sufficient involvement of the general public and that there was a need for greater engagement of a wider range of stakeholders, including those from other countries.

Dr. Selgelid felt that some policy decisions, especially on risks affecting the global community, could only be made by an international body. He suggested that GOF research poses issues of global justice, including sharing of the benefits of this research. If the risks are universal, there may be issues if the benefits are available only to some of the countries. Dr. Selgelid felt this was particularly important for medical countermeasures. He also noted that a decision to conduct GOF studies of concern in only maximum-containment facilities would effectively preclude the majority of countries from undertaking such work. Dr. Selgelid suggested that WHO was the most legitimate international body to make decisions about GOF research. He discussed the possibility of creating a new WHO committee, similar to the body that oversees smallpox research, to undertake such a task. Dr. Selgelid also discussed the possibility of developing a new stand-alone body for the oversight of GOF studies of concern.

Dr. Selgelid noted that some countries are more likely to be exposed to risk from GOF research than others, especially where vaccines or therapeutics available in richer countries are not available in poorer countries. He noted that differences in access to basic healthcare could also result in an uneven risk distribution from GOF studies of concern.

He highlighted a number of lessons from earlier discussions of DURC. For example, he reviewed findings from the 2010 WHO guidance document *Responsible Life Sciences Research for Global Health Security* (WHO, 2010) (see Box 4-5). Dr. Selgelid also identified a number of approaches for harmonizing GOF research policy. He noted possibilities for gathering greater input from other countries bilaterally. He discussed a collective international harmonization process to create a level playing field. He also considered a more formal international governance regime for policy making and decision making. Dr. Selgelid reviewed the possibilities for using different frameworks, either by strengthening existing treaties or by creating new international agreements or compacts. He cautioned that these more formal arrangements would be difficult to achieve and involve a great deal of work. He also noted more standards-based approaches to

BOX 4-5
Insights for GOF Policy Making Drawn from Past Discussions on Responsible Life Sciences Research for Global Health Security

- There will not be one-size-fits-all approaches to managing risks—some solutions make more sense in some places than others.
- Opportunities to leverage existing regulations and governance structures should be explored whenever possible.
- If additional measures are needed to deal with GOF, expanding existing committees overseeing research might be considered rather than building new ones.
- There are different levels that can be used to address concerns over research: some approaches can be undertaken by individual scientists; other activities can be conducted at the institutional level; professional scientific bodies need to make certain decisions (such as on codes of conduct); other decisions need to be made by domestic governance (such as on regulations or education); and funding bodies can play an important role by taking risks into account when making funding decisions.

SOURCE: Selgelid, 2016.

governance, discussing the framework in place governing human subjects research and suggesting that the ethics governance regime might be expanded to include oversight of GOF studies of concern.

Discussion

The discussion that followed expanded on ideas and concepts introduced during the presentations. Participants explored the comparative advantages of using a standards-based approach based on the existing ethics governance regime. Michael Selgelid again argued this might be easier than a treaty-based approach because it could take advantage of existing policy frameworks and offer logistical benefits. Participants also discussed whether international harmonization might be best achieved through international organizations or international scientific bodies. Some participants felt that both approaches should be pursued in concert. Opportunities for using insurance requirements to harmonize GOF approaches were also discussed, and David Stanley introduced a concrete proposal from the Future of Humanity Institute to utilize the grant making process to address potential risks (Cotton-Barratt et al., 2016). The institute proposed to "price the expected value of any damages that could result from GOF research into the price of the grant being considered.

Then they could either require grantees to purchase liability insurance to cover the possible damages from this or, alternatively, require a payment to the state or non-state body to cover the expected cost of that research."

The reasons for seeking international input and harmonization were explored. Christopher Park from the Department of State outlined three objectives for seeking greater interaction, including (i) to get greater clarity as to foreign views on U.S. measures; (ii) to change behavior of individual researchers, perhaps best achieved through international scientific bodies; and (iii) trying to change behavior of other governments, requiring different approaches either through multilateral settings or coalition building. He also noted that if the intent was to address laboratory biosafety issues, it would require engaging one set of actors in associated settings, while a separate community and associated forums would be necessary for addressing biosecurity information risks. Another participant highlighted the importance of engaging the human and animal health communities, given the zoonotic nature of relevant diseases.

Keiji Fukuda from WHO stressed the importance in successful international efforts of a common understanding of the nature of the risk being addressed. He offered the negotiation of the International Health Regulations, the WHO Pandemic Influenza Preparedness Framework, and measures to address antimicrobial resistance as examples. He then suggested that such an international common understanding does not exist with regard to GOF studies of concern and that international engagement might be better focused on reaching a technical agreement on the nature of the risk posed by this research.

Participants also discussed three options for balancing national action against a broader international approach: to act now solely at a national level; to act now at a national level but send a clear message as to the desirability of subsequent international engagement; or to begin working on a full international policy process from the outset. Several participants felt that, given the international nature of the risks being addressed, the first option was not appropriate for GOF research. The same participants suggested that the decision as to whether the second or third approach was more suitable should be based on the resources available, the preexisting levels of international concern, and the level of need for international consensus. Another participant suggested that if sufficient resources could not be secured from the outset, it might be better not to initiate an international process rather than have to abandon it after a short while. Some participants highlighted the value of the Global Health Security Agenda as a model for building an international partnership with opportunities to shape the process.

One question raised during the discussion was who should determine the criteria for classifying research as GOF studies of concern or

for identifying specific research proposals that meet those characteristics. Dr. Selgelid suggested that it might usefully be based upon a mutlilayered analysis with institutional, national, and then international stages—relevant research would be identified at each of these levels and then passed on to the next level for further consideration.

The potential for additional oversight measures for GOF studies of concern to reduce interest in GOF research was raised again. Some participants pointed out that in some cases, such as certain types of research involving human subjects, this was acceptable and appropriate. Gabriel Leung suggested that the longer-term impact would be to discourage scientists from entering into research fields connected to emerging or re-emerging pathogens. This was disputed by others, such as Marc Lipsitch from Harvard University.

Participants also discussed possible reactions by international partners, such as China, if the United States decided to introduce an oversight framework for GOF studies of concern. George Gao felt that China would certainly look closely at such a regime.

5

Summing Up

Harvey Fineberg, chair of the Symposium Planning Committee, explained the plan for the final session. First, he would ask the moderators from the various sessions, all members of the planning committee, to offer their perspectives, summarizing and perhaps adding their own personal comments about key points that were raised in each of the sessions. He then wanted to allow an opportunity for those from the National Institutes of Health (NIH) and the National Science Advisory Board for Biosecurity (NSABB) to raise any issues or topics or questions they would like to address, and to invite further comment from those on the stage and in the audience. Finally, the microphones would be opened for additional comments, suggestions, and ideas that anyone present or listening on the Web would like to include in the record.

Dr. Fineberg invited Charles Haas ("Informing the Policy Framework: The Risk and Benefit Assessment") to give the first summary comments. Dr. Haas began with one editorial comment. Data gaps, particularly on laboratory safety, were thought to limit the ability to do an absolute risk assessment, and there had been a good set of questions from the floor about the need to develop scholarship and support for those studies. His comment was that such data are not totally absent, and it might have been informative to use whatever data were available, even though they were poor, as part of the effort to bound the potential risks that could occur.

Dr. Haas also commented that, if a pure "risk acceptable" rule is to be used as a basis for decision making, it should be recognized that information is lacking on what the level of acceptability should be. Rocco

Casagrande had presented an updated analysis using new data on seasonal versus 1918 influenza, which raised the broader point that risk assessments in general need to be living and to be adaptable to new information as it comes along. Dr. Haas also cited Adam Finkel's statement that leaving uncertainty out is a violation of first principles.

He quoted Dr. Finkel that "Is it safe?" is a vapid question because it is intrinsically without meaning without a reference level. A hierarchy of potential judgment rules exists. Both Tony Cox and Dr. Finkel made that clear, and also that explicit judgments about what rule is to be used need to be made. Kara Morgan called this "deciding how to decide," and she noted that there is rich scholarship from the decision analysis community that needs to be brought to bear. And stakeholder input needs to be included to develop the decision rules.

Dr. Cox had cited the need to avoid the "fallacy of coherence": Just because risk has been accepted in the past does not mean that an informed judgment going forward would make that same numerical risk acceptable. A useful task would be to assess whether or not collection of more information would make a decision better. There is a rich literature on the concept of the value of information in this regard.

Dr. Haas concluded by citing a number of miscellaneous problems that had come up in the discussion. For example, Dr. Casagrande had expressed the concern that bench researchers may not be familiar enough with epidemiological parameters to assess transmissibility. Next, risk–benefit analysis could be used to improve the risk profile of proposed experiments—in other words, envisioning an iterative process of some sort. Dr. Finkel had argued that risk and benefit analyses should be balanced, humble, and explicit about value judgments. And finally, there had been comments from the audience that particularly long-term benefits may be difficult to value and highly uncertain. His editorial comment in response was that, while this may very well be true, it should not mean that one should walk away from the effort to attempt to quantify them using whatever information one had available.

Barry Bloom shared reflections from two sessions, first on behalf of Michelle Mello ("The Policy Landscape: United States") and then from the panel he had moderated ("The Policy Landscape: International Dimensions of Gain-of-Function [GOF] Research"). Dr. Mello's comments included:

- There is no set of policies that targets the specific group of pathogens defined by the NSABB. Instead, the federal policy framework consists of a series of partially overlapping statutes and regulations that are largely tied to specific pathogens and to federal research funding. None of the panelists pointed to major gaps in this framework other than noting that the Department of

Health and Human Services targets a *very* narrow set of experiments and the dual use research of concern (DURC) policy covers only 15 pathogens. However, their comments did reinforce the NSABB's observation that the strength of the policy oversight is stronger for some pathogens than for others.

- Existing law does not really reach research that is not conducted with federal funding (i.e., industry-sponsored research). This raises the question, should it? And if so, through what mechanism?
- The time to regulate is at the time the research is conceived. The point of publication is far too late. Having a strong review process up front avoids a lot of problems down the line—and also establishes that institutions have acted with due care (which may come up in litigation). Funding agencies and institutions can engage principal investigators (PIs) at the point of designing their protocols to think through the risk issues. This is especially useful because many PIs do not understand dual use risk issues.
- Regulators, including both institutions and federal agencies, can benefit from greater use of consultation. Talking with each other and with external experts can boost the quality of review and the dissemination of knowledge and best practices.
- Epistemological question: How do we know if a regulatory approach is working? Beyond the absence of rare, catastrophic events, what should we use as performance measures? The panelists suggested public trust, but in her view, this is both hard to assess and a narrow measure. The NSABB may wish to think (in relation to its Key Finding 2) about what it means to say the policy frameworks are "effective."
- One tension in oversight is between the desire for transparency and the risk that public disclosure of sensitive information will elevate the very dual use risks that oversight is aiming to minimize.
- The criteria that the NSABB set forth for reviewing GOF research are reasonable, but not very specific. They rely on subjective judgments such as "likely" and "highly." Yet there is a tension between pursuing greater specificity in regulations and providing enough flexibility to make case by case judgments. Also, it is not clear how to get more specific about some of these standards.
- How much variation should be tolerated in how institutional review committees evaluate research? On the one hand, one would like to have common standards applied in a reliable fashion. On the other hand, institutions have different capacities, and there might be something one can learn from their individual innovations in practices. The panelists did not see a major prob-

lem with having a "patchwork of institution-dependent rules"; this is something the NSABB may wish to consider.

Dr. Bloom then turned to the comments on the session he had moderated ("The Policy Landscape: International Dimensions of GOF Research"). It was clear from the very beginning of the sessions on the first day that everyone involved in this meeting recognizes that science and the risks and benefits have global implications, and GOF research clearly has raised global concerns. The session included major presentations on the groundbreaking progress made by the European Union (EU), which showed that it was possible to have discussions and bring policies from 28 countries to a common focus, and bring scientific academies in almost all of those countries to a consensus on the scientific policies that would govern this research. The discussions emphasized the need to expand and extend the discussion among countries in Europe. The panelists would be very interested in discussions after the U.S. policies are formulated, and they were eager to find out ways in which discussion and consultation can be expanded to include all countries.

In this context, the session heard a very important discussion of the InterAcademy Partnership, a global network of science and medical academies that now links academies in 128 countries and four regions. That could serve as a useful focus for extending the discussions of GOF research in a coherent way to responsible scientific bodies that already exist and perhaps should be considered in moving forward.

A suggestion that emerged from the session was that the best place to start is probably with discussion within the scientific community rather than going directly to policy makers one at a time, one country at a time, until there is some general understanding and agreement within the scientific community. Then the complexities of those dialogues and discussions could be simplified to a level that could gain understanding and support from political leaders.

The session also heard about the value of not just pontificating but having important partnerships and collaborations that enable transparency, technology transfer, and training to occur. These can also be a way of maintaining standards and identifying low standards that need to be addressed.

He offered several personal reflections about what he had learned during the meeting.

- He had come to the view that process is probably as important as principles. It is not clear, given the technicalities of the science, that the lay public, and even government officials, are going to understand the technicalities. But if the processes at every level

are transparent, maybe that is the best way to gain trust within the scientific community and within the public at large. And that means the processes as he was conceiving them, and as the NSABB conceives them, are a set of tiered processes that occurs at multiple levels from the investigator, the Institutional Biosafety Committee (IBC), the institutions, study sections, and all the way up to the higher levels of policy.

- His second reflection on the meeting is that whatever one does, it has to be recognized that science is changing dramatically so that policies cannot be fixed in time to predict what possibilities, opportunities, technologies, and threats will be coming in the future. The policies need to be flexible in some way to accommodate new knowledge and adapt to new opportunities and possibilities and yet have a clear-cut framework that people can work with.
- Finally, he supported Gabriel Leung's comment about why the Biological Weapons Convention (BWC), as far as we know, largely works. Why do the Helsinki principles actually govern how human experimentation is done? He would say it is less legal liability and lawsuits than it is to ask, what are the principal constraints on scientists? He believed those have to do in general with constraints on reputation, credibility, integrity, and respect in the scientific community. Matthew Meselson, for example, when asked how one could possibly encourage more action to enforce the BWC, raised the interesting possibility of making it impossible for scientists who violated international law to travel overseas as another constraint that would be of high value for scientists. So he believed enforcement at a moral level is highly possible.

Baruch Fischhoff offered his comments on the session devoted to "Informing Policy Design: Insights from the Science of Safety and the Science of Public Consultation." He began with some nomenclature, using the term "social science" for those not familiar with that part of the world to include social, behavioral, and decision science. Behavioral science is the study of individuals; it is psychology, microeconomics, neuroscience, and other social sciences. For larger groupings, it is sociology, anthropology, and political science. And decision science is management science, the cost, risk, and benefit analysis of that form of applied mathematics that takes human behavior into consideration. For problems of this complexity and subtlety, he argued that insights from all these fields are needed.

The framing of the human dimensions that he believed came out of the session is that reducing the risks and realizing the benefits of these technologies depends on people at the level of individuals, organizations,

and policies. Second, relying on intuition in designing and evaluating the systems that deal with these technologies is natural, but it is unfortunate because those intuitions are often wrong or imprecise. Third, the biological research community faces the challenge of not having what some economists call the absorptive capacity for social science. That is, there is nobody on the inside who can tell when they have a social science problem, define it in terms that would be recognizable to a social scientist, and find somebody who will help them to work on the problem. That is on the demand side. On the supply side, the social science community may lack the incentives for addressing biological science issues because its incentive scheme is to publish on relatively narrow topics. He thought the symposium was fortunate to have speakers in his session who have that bridge which requires them to draw on different social sciences as well as to see the value for the basic science to engage in applied problems.

He then asked what kinds of issues one would find if one brought the social sciences to bear? One is to identify the places in which scientific judgment affects the prediction of outcomes. Many of the statements heard during the symposium had to do with scientists anticipating how transmissible something would be. Given that this a discovery process, there are likely to be surprises. So it is smart to recognize that these are scientific judgments and to elicit them in the best, most accountable way possible. Second, these are ethical judgments and analyses: for example, how you define them, who you share them with, where various publics are engaged in the process. Third is the communication to and from stakeholders so that one can develop the technologies in the ways that are most sensitive to their needs and keep them properly apprised of developments.

A fourth problem, more from the social sciences, is the normalization of pathology and the virtue. One can become accustomed to best practices that are terrible by any absolute standard. But as Ruthanne Huising's talk and Dr. Bloom's comments illustrate, there is also the possibility of the normalization of virtue. There are things that one just does not do, and this is part of the kind of bottom-up process of acculturation and socialization that Dr. Huising discussed.

Fifth, there can be a mismatch between the technology and the regulatory mechanisms in terms of not just government regulation but also the societal controls that one has over technologies. One can have regulatory control mechanisms that do not have the requisite variety for technology that is moving very quickly when institutions were developed for a different environment. Another problem that one runs into is the neglect of opportunity costs. A good deal is known about the technologies in which one has invested and much less about the ones in which one has not invested.

Dr. Fischhoff concluded, in the spirit of Dr. Bloom's two personal comments, with two recommendations.

- Given the difficulty of bridging the basic and social sciences, there would be value in creating centers that would serve as a kind of clearinghouse for helping interested biologists to find social scientists who could help them work with their problems and social scientists to find the people with whom they are willing to work. They could help make the case to department heads that this is a worthy pursuit to spend as much time as all three of the speakers have had working with clients to apply the social science that is available and to create the needed evidence for what some people call adaptive management.
- The second is to develop shadow alternative evaluation processes. That is, if current mechanisms are not up to it, alternative mechanisms are needed. Monica Schoch-Spana's talk illustrated the potential to bound the set of deliberative mechanisms whereby this might work. But one will not really know how they would work until people with the different kinds of expertise and cultural experiences come together and explore them. And one might hope that if there were some worked examples—maybe like some of the conventions that people have talked about—they would eventually become the normal thing that people do. It is very hard to get people to repeal regulations that promise safety, but sometimes they just atrophy. And maybe they will go away if we have something better.

Philip Dormitzer offered his reflections on the ideas raised by what Dr. Fineberg called the "interested parties" in his session ("Best Practices to Inform National Policy Design and Implementation: Perspectives of Key Stakeholders in the Biomedical and Public Health Communities"). He began with Michael Callahan, who pointed out that the European Union and the United States are not the future epicenter—and may not even be the present epicenter—of GOF research. And similarly, government funding may not necessarily be the dominant mode of funding for this research. It is necessary to expand the thinking about how one might influence these processes. Another very interesting point was that some of the case studies he offered where mechanisms of control of infectious agents of concern were lost not due to any malicious intent but due to the necessities facing people operating under difficult circumstances. There are circumstances where consultative mechanisms might help, where forms of assistance might help, and also where incentives need to be created to encourage people to limit risks when there is no capacity to regulate their behavior.

Robert Fisher had discussed the inherent conflict between the need for evidence-based decision making at the regulatory level, which is necessarily time consuming and expensive, and the frequent need to act quickly, particularly in these emerging or outbreak situations. This conflict has to be reconciled, and the considerations around policy for GOF studies of concern play into that. And this also raised the earlier point that estimation of risk can really only be judged in a context of expected benefit. Without benefit, why would one take any risk? These things play into the sorts of mechanisms that one might pursue to try to control the risks of GOF studies of concern.

Dr. Dormitzer commended Jonathan Moreno for trying to identify where there are areas of consensus regarding policy for GOF research. He did not know if everyone agreed on those areas of consensus, but he thought they were close enough to be worth mentioning. There is consensus that there are times when it is necessary to move quickly, but also that some regulation is needed. There is consensus that biocontainment is imperfect, that risk mitigation heavily involves human factors, especially as the mechanical and environmental factors get under better control. He thought that there was consensus it would be desirable to have alternatives to risky experiments, and that gain of function experiments are not fully predictable, but the capacity is probably improving.

Dr. Moreno also had a very interesting proposal for what he called R-BATs, or Risk–Benefit Assessment Teams. The idea is that there would be real-time, ongoing, interactive evaluation of experiments of concern or experiments that may not yet be of concern but could venture into that area so that there was not simply a checkpoint—for example, at the time of funding and another at the time of publication—but an ongoing process of interaction. Dr. Dormitzer thought that might not take care of the whole issue, but it could make a very solid contribution.

Finally, Ethan Settembre had discussed some of the lessons of the first H1N1 pandemic in 2009 and then the H7N9 outbreak response in 2013, making the point that GOF research is an inherent part of the routine business of vaccine production. Unintended consequences of GOF policy choices therefore needed to be considered.

Dr. Dormitzer noted that today sequence analysis is a part of risk analysis and vaccine virus selection, but it is secondary at this point to phenotypic, clinical, and epidemiologic characterizations. He thought, however, that will start to shift over time. It is certainly never going to be the case that a sequence analysis can replace current approaches, but the volume of relevant sequence data is likely to increase dramatically. It is now possible to sequence flu strains directly from harvested secretions; there is no need to grow the virus. The ability to do that sequencing is becoming increasingly widespread, and it is quite con-

ceivable that these will be done in some sort of handheld devices in the coming decade.

Dr. Dormitzer closed with some personal observations. One was an increasing need to consider integration of the multiple biosafety and biosecurity regimens. The other was a concern about unintended consequences: for example, from the "blowback" onto vaccine production from the controversies over GOF studies of concern—or GOF research more generally—in academia.

Ronald Atlas began the discussion of the session he had moderated ("International Governance: Opportunities for Harmonizing GOF Research Policy and Practice") by remarking that he had learned that the international dimensions of the debate about GOF research, risks, and benefits cannot be ignored. A number of possible ways of approaching that on an international scale had been suggested. One was to go to a non-regulatory framework to take ethics or other sorts of systems that have gained traction and are accepted across the biomedical field, build on those, and essentially build a culture of responsibility within the community that would assure the public that everyone was taking the appropriate mitigation steps. Another was to simply accept that nations that were carrying out GOF research would develop their own sets of regulatory frameworks. Another was to allow the efforts that are ongoing in areas like the United States and the European Union to begin to cross-fertilize each other and to bring together groups that would then allow for voluntary harmonization without going to an international organization like the World Health Organization (WHO). And finally, the higher level is to go to a United Nations agency such as WHO and attempt the perhaps impossible task of coming up with a global regulatory scheme.

Dr. Atlas thought that another important point from the session came from Keiji Fukuda: the need to find a compelling and readily understood reason to come together at the international level to take action. What would that reason be for GOF research? Dr. Atlas suggested that it could be "preventing a global pandemic." That could mean that the research is absolutely necessary because it will provide the vaccines, the surveillance, or whatever to prevent the pandemic. Or to take the opposite side, the research itself is a risk because something could get out and cause a pandemic. That is the dilemma underlying the entire debate over GOF research, and he was still not sure there would ever be an answer that was satisfactory to everyone.

Dr. Fineberg then asked if any NSABB members had comments or questions. Joseph Kanabrocki from the University of Chicago and co-chair of the NSABB WG began with some observations. He was heartened that the comments and discussion suggested that the NSABB had not made any major missteps. He was also pleased that there was movement away

from a list-based system to a phenotypic system that the NSABB has been recommending for a number of years. That had not been explicitly stated but he thought it was implicit in the discussions.

Dr. Kanabrocki said that, speaking personally, he had heard a number of things on which the NSABB WG had not yet deliberated that he would like to see added to the NSABB report. These included incident reporting mechanisms that could address the lack of data highlighted by the risk and benefit assessment as well as the need for harmonization, both on the national level and the international level. He thought it should be something the final report called for more explicitly, and addressed some of the ideas about how that could be accomplished. He also hoped that the NSABB would recommend a code of conduct for scientists engaged in this type of research.

Dr. Kanabrocki then returned to the three phenotypes recommended in the draft report as the criteria for identifying GOF studies of concern. The original version of the NSABB WG's Draft Working Paper included resistance to countermeasures as an example. He stressed that it was intended only as an example, but, unfortunately, people seemed to have seized on it as the one aspect of the third criterion. So he wanted to remind everyone that for him—and he thought most of the NSABB as well—the third phenotype is what makes this an issue of pandemic potential. He thought the first and second traits go to the animal pathogen interface, and the third trait is where one addresses human public health, the societal aspects of pandemic. He thought that the third trait remains critical, though it might be possible to revise the language in a way that is more palatable.

Susan Wolf, an NSABB member from the University of Minnesota, raised the issue of oversight design and said she wanted to try out two ideas, one at the institutional and one at the federal level. This is crystalized by the flow chart introduced at the symposium (see Figure 2-2). The NSABB has developed the chart to communicate visually the oversight process it is planning. At the institutional level, who decides that an experiment is a potential GOF study of concern? At the moment, the NSABB is envisioning the initial determination would be made by the PI and the local oversight authorities, presumably the IBC. Her concern was how to avoid recapitulating the history of Institutional Review Boards (IRBs), which she characterized as being very slow to design, much less to put in place the sort of "learning" oversight system where there is a systematic effort to gather experience and share lessons learned and also to identify unjustified variations in how the rules are applied. There is a substantial amount of research on this problem and she hoped it would be applied to ensure that the GOF system would be state of the art.

Her other concern was at the federal level and what would happen if a GOF study of concern is identified at the local level. Who would review

it and apply the several principles the NSABB was proposing? Could one answer be a new Federal Advisory Committee Act (FACA) committee charged with this task?

Marie-Louise Hammarskjöld, an NSABB member from the University of Virginia, asked about the issue of how to capture research done without federal funding, citing increased interest from industry in university research. She thought that, given that the concern was potential pandemic risk, the board might not be doing its job if it did not deal with that part of the research enterprise.

Jim LeDuc, an NSABB member and Director of the Galveston National Laboratory at the University of Texas Medical Branch, was particularly interested in risk mitigation. His question to the panel was how to create a foundation upon which a policy can be built that clearly articulates the requirements for biosafety and biosecurity, and importantly, a culture of responsibility that spans the scope from the individual scientist all the way through to the institutional leadership.

Dr. Atlas reacted to the question of the IBC versus the national level and suggested that a great deal was learned during the early days of the Recombinant DNA Advisory Committee (RAC). He commented that the IBCs sent cases to the full national board until the RAC was able to demonstrate to the local IBCs what was and was not of greater concern. The RAC refined the principles, and he thought the same approach should be taken for GOF studies of concern. What is needed is to create a learning process, an iterative process, where there is appropriate consultation from the national back to the local and eventually the local learns how to handle the cases and the burden on the national board diminishes.

The RAC had also dealt with the question of federal funding. It turned out that the first cases that came to the RAC were from industry, which wanted the national approval. Industry did not want to go around the system; it wanted to become part of the system even though it was not mandated to do so. He had no reason to think the same thing would not happen here.

Dr. Dormitzer said that he could certainly speak for having been in companies when there are national and accepted standards. Even when not required to follow them, companies in general want to do so. In fact, the most distressing situations are those where there is a lack of clarity over what the expectations are. And that is why the ideas about advisory boards and groups to which companies can turn to ascertain what those standards are, even if compliance is voluntary, are useful. He thought there would be a widespread desire to meet the standards.

Dr. Fineberg added a comment about the discussion of the importance of the scientific community building and reinforcing a culture of safety as well as a discussion about the importance and practicality of public

engagement and about the various types of publics. It seemed to him that in the thinking of the NSABB, going forward it would be useful to consider a model that incorporates, at an appropriate level, a FACA-like entity and relevant public participation as a way of building the kind of larger trust, and, frankly, reinforcing the community of safety, both within and around the scientific community, on which success ultimately will depend.

Dr. Fischhoff commented that he was involved with the Food and Drug Administration (FDA) over the past few years as the Center for Drug Evaluation and Research developed a benefit–risk framework (FDA, 2013). The framework was developed jointly with its staff and resembles Kara Morgan's model of deliberative criteria-based frameworks (see Box 2-3). It was designed to help people tell their story in a way that one could see what the logic was; one could compare across decisions; and one could find the decisions that were—as someone has mentioned—anomalous and that gave industry a clearer sense of the kind of things that the FDA was approving.

Dr. Fineberg made another observation on the first and fundamental question of the phenotypic inclusiveness or exclusiveness. One of the things he heard repeatedly in the course of the discussion was the importance of circumscribing the domain of concern so that neither the scientific community nor the regulatory authority, nor, frankly, the interested publics were needlessly burdened with a wide variety of questions that truly do not raise and rise to a level of concern. At the same time, there was a lot of discussion as to whether the current formulation—where the requirement is that a given experiment affects all of the elements—is a sufficient degree of circumscription. He thought that the real challenge for the NSABB was to reflect its actual intent in its description and to do so in a way that is clear and understandable over time. So, for example, he thought that one could be overly fixed on the models that depend on familiarity with influenza as the case. He thought the policy that will be promulgated ultimately needs to be capable of dealing with GOF research, and increasingly, experiments that intend to develop entirely novel organisms with capacities and capabilities that are not currently even expressed in existing microorganisms. And if one thinks that broadly, defining a phenotypic space that involves virulence, and involves transmissibility, and involves resistance to treatment, if that is how one wishes to characterize it, one could imagine placing imaginably any organism at a point in space that has those three attributes defined. Thought of that way, there is an aspect of this space where one would not want research to go at all. There is an aspect of that space where one would not want to require further review. And then there is an aspect of that space, depending on the starting point and the direction of the experiment to make it worse or to make it

better—and this is where vaccine development comes in so importantly—would dictate that it may, then, be a topic that requires consideration as a GOF study of concern. He said he hoped that it would be possible for the NSABB to mull over this question and to think about ways to characterize and describe exactly what it believes should determine a consideration for GOF studies of concern. And, perhaps, to be explicit about excluding vaccine development research, which is so fundamental to protection and actually contrary to the concerns. And to be able to apply the principles more generally as new ideas with different organisms will naturally arise in the creative minds of scientists.

Dr. Kanabrocki agreed and said that he wanted to clarify again that, as his NSABB WG co-chair Ken Berns had said on the first day, the NSABB was not really worried about what goes in, but what comes out. The NSABB WG was not saying that the experiments of concern are only those that would result in the three phenotypes. What they were saying is the experiments of concern are those that result in an organism that displays those three phenotypes, and there is a difference. Because one could begin with two of the three and contribute the third and that would be an experiment of concern.

Dr. Fineberg then opened the floor to questions and comments from the participants. Wendy Hall from the Department of Homeland Security asked a question in terms of precedent. First, how important is it that one has full awareness of the GOF experiments being proposed throughout a variety of different labs in the United States? She was not sure there is clarity across the academic community at any one point in time about who is planning and doing what. Her second question related to the experience with the Select Agent rules, which were implemented in 300 various labs with a substantial range in the quality of performance. In GOF research, is there any precedent—if the academic community had full visibility, peer to peer, institution to institution—that there could be corrective elements from the institutional bodies with each other to redirect or help labs not performing as well? Her hope was to avoid the need for the government to have to come down with tough, restrictive language across the board that affects everyone because of a case where one or two labs make an error that makes the mainstream press.

Dr. Fineberg responded that her question reinforced the importance of the scientific community itself coming together in a coherent way on this and related issues of safety and security. From a personal point of view, he did not think the government alone could accomplish this, nor could the community, acting without the guidance of shared standards. So he thought the efforts would be mutually reinforcing.

Dr. Schoch-Spana from the Center for Health Security of the University of Pittsburgh Medical Center picked up a point that Marc Lipsitch from

Harvard University had made about the capacity for innovation, not just prevention. Are there things, such as special research funds, that could incentivize scientists to try alternative approaches to GOF studies of concern? If systems are put in place and data are gathered about the kinds of experiments that are not funded, those data could be synthesized to identify lines of work that need to be replaced with safer alternatives, and research to develop those alternatives could be eligible for special funding.

Nicolas Evans from the University of Pennsylvania offered two comments. The first concerned the Declaration of Helsinki, which was a great initial work in establishing norms in human subjects research and biomedical ethics. But he thought that the FDA's removal of the Declaration of Helsinki from its regulations was an indicator that, as a model for governing the life sciences, one should be especially careful about the way one seeks international collaboration. If the United States sets up or attempts to initiate other arrangements for governing GOF research, only to pull out of them because it does not want them referenced in its own legislation, that would pose a major problem. He also built on Dr. Lipsitch's and Susan Wolf's comments about the critique that IRBs and biomedical ethics chill biomedical research, commenting that it had been made many times and citing two recent works (Klitzman, 2015; Schneider, 2015).

Dr. Evans also offered three other comments.

- He thought it was very important conceptually to make a clear distinction between general GOF research, which is accepted as a valuable and commonly used technique, and specific GOF experiments resulting in the creation of novel pandemic pathogens that is beneficial. For example, the Gryphon Scientific benefits assessment had concluded that a portion of the studies it assessed provided unique benefits.
- Dr. Evans noted that health care workers, the people who bear the disproportionate burden of risk in the event of an infectious disease outbreak, had been entirely absent from the discussions.
- Regarding innovation, he commented that because $820 million had been provided to synthetic biology research over the past half decade, it seemed prudent to also spend a small amount of money on innovation in applied biosafety, such as on material science to improve personal protective equipment.

Jenna Ogilvie from the National Academies of Sciences, Engineering, and Medicine's staff brought two questions from the Web. The first was from Grigory Khimulya from Harvard College. Do current oversight frameworks provide adequate treatment of novel pathogens that

were never seen before and are not on the pathogen lists mentioned in the NSABB's draft recommendations? For example, if a new potentially pandemic pathogen like Middle East respiratory syndrome (MERS) is identified, would GOF studies of concern with this pathogen fall under proposed regulation? The second question, to Dr. Casagrande from Gryphon Scientific, came from John Kadvany from Policy & Decision Science in Menlo Park, California, prompted by publications suggesting that GOF research has characteristics of so-called potential "normal accidents," in which a technology combines highly negative outcomes (e.g., a nuclear plant meltdown) with unquantified and perhaps unquantifiable scenarios falling outside even the most complete probabilistic risk analysis. Gryphon Scientific's work suggests that such scenarios may be relevant with the extreme negative outcome being pandemic risk. Did Dr. Casagrande have an opinion on this characterization of GOF studies of concern? Is it correct in some respects as it may be for some contemporary technologies? Or is there a characterization fueling clashing GOF risk perceptions?

Dr. Casagrande commented from outside his role as PI of the risk and benefit assessment to push back a little bit on several comments he had heard about what could be learned from the successes of the BWC. He thought that the protocol was a better exemplar because it banned first use of bacteriological warfare. In contrast, several members of the BWC have violated its provisions, leading him to conclude that one ought to learn from its failures, such as the lack of a verification and inspection regime and the lack of an enforcement capability that is relevant internationally.

Dr. Lipsitch from Harvard University commented that there had been considerable discussion about whether there is consensus that there are any experiments that everyone would agree would never be acceptable and any experiments everyone would agree should never be impeded. He said he could certainly think of experiments and developments one would never want to impede and suggested that there should be a green line as well as a red line. He thought that whatever regulatory framework or oversight framework is developed, it would be incredibly helpful to have at least those two kinds of cases spelled out by some examples in order to build our intuition for the next time something comes up that is not envisioned yet. He also thought some more contestable case studies, where there would not be an easy consensus, would be useful.

Dr. Kanabrocki responded to Dr. Lipsitch. The NSABB WG had tried on a number of occasions to think of experiments that absolutely should not be done. And every single example that came up was of an experiment that lacked scientific merit. So he suggested that, in his personal view, it would be a struggle to think of experiments that have scientific merit that should not be done.

Gerald Epstein from the Department of Homeland Security suggested that it would be useful to go back to the Department of Health and Human Services (HHS) framework that Larry Kerr had described on the first day, and the test that a proposed project would have to satisfy before it was deemed acceptable for funding. One was that the pathogen to be constructed was one that might occur by a natural process, so that there was a reasonable expectation nature might get there first. If it is not something nature might do on its own, one could not argue the work was to defend against a potential natural development. This might be an example of something on the other side of the line, at least from the precedent of the existing HHS framework.

Dr. Fineberg closed the session by expressing the Academies' deep appreciation to everyone who had taken part, in person or via the Web. He commended the work being done in Europe and commented that, in his view, a policy about GOF research that applies only to one country is not a policy that will work for the safety of the world. And that is something of which one needed to be very mindful. He also commented that it was evident from all the discussion that whatever the next iteration of conclusions and recommendations that emerge from the NSABB is, it will really be one step in a process that is likely to continue. It will require continued refinement, the engagement of the scientific community, and finding creative ways for the public that is interested and affected by GOF research to be involved in the process of decision making going forward.

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Appendix B

Committee Biographies

Harvey V. Fineberg (*Committee Chair*) is the president of the Gordon and Betty Moore Foundation and served two consecutive terms as president of the Institute of Medicine (IOM) (2002-2014), now known as the National Academy of Medicine. He served as provost of Harvard University from 1997 to 2001, following 13 years as dean of the Harvard School of Public Health. He has devoted most of his academic career to the fields of health policy and medical decision making. His past research has focused on the process of policy development and implementation, assessment of medical technology, evaluation and use of vaccines, and dissemination of medical innovations. Dr. Fineberg helped found and served as president of the Society for Medical Decision Making and has been a consultant to the World Health Organization. At the IOM, he chaired and served on a number of panels dealing with health policy issues, ranging from AIDS to new medical technology. He also served as a member of the Public Health Council of Massachusetts (1976-1979), as chairman of the Health Care Technology Study Section of the National Center for Health Services Research (1982-1985), and as president of the Association of Schools of Public Health (1995-1996). Dr. Fineberg serves on the board of the Hewlett Foundation and chairs the board of the Carnegie Endowment for International Peace. Dr. Fineberg is co-author of the books *Clinical Decision Analysis*, *Innovators in Physician Education*, and *The Epidemic that Never Was*, an analysis of the controversial federal immunization program against swine flu in 1976. He has co-edited several books on such diverse topics as AIDS prevention, vaccine safety, global health, and understanding risk in

society. He has also authored numerous articles published in professional journals. Dr. Fineberg is the recipient of several honorary degrees and the Stephen Smith Medal for Distinguished Contributions in Public Health from the New York Academy of Medicine. He earned his bachelor's and doctoral degrees from Harvard University.

Ronald M. Atlas is professor of biology at the University of Louisville. After receiving his master's and doctoral degrees from Rutgers University, he became a postdoctoral fellow at the Jet Propulsion Laboratory where he worked on Mars life detection. He has served as chair of the National Aeronautics and Space Administration's Planetary Protection Subcommittee, co-chair of the American Society for Microbiology (ASM) Task Force on Biodefense, and a member of the Federal Bureau of Investigation Scientific Working Group on Microbial Genetics and Forensics. He also served as president of ASM and was a member of the National Institutes of Health Recombinant Advisory Committee. He currently chairs the Public and Scientific Affairs Board of the ASM. His research has included development of detection methods for pathogens in the environment. Dr. Atlas is author of nearly 300 manuscripts and 20 books, and regularly advises the U.S. government on policy issues related to the deterrence of bioterrorism.

Ruth L. Berkelman is the Rollins Chair and Director of the Center for Public Health Preparedness and Research at the Rollins School of Public Health at Emory University. She holds appointments in the departments of epidemiology, global health and medicine, and serves as a senior associate faculty member in Emory's Center for Ethics. She previously served as an assistant surgeon general in the U.S. Public Health Service at the Centers for Disease Control and Prevention (CDC). Elected to the Institute of Medicine (now the National Academy of Medicine) in 2004, she has served on various committees, including the Forum on Emerging Infectious Diseases and the Board on Life Sciences. She has been a member of the National Biodefense Science Board and the Board of Trustees at Princeton University. She was previously Chair of the Public and Scientific Affairs Board of the American Society of Microbiology. She currently chairs the Board of Scientific Counselors for infectious diseases at CDC.

Barry R. Bloom is a leading scientist in the areas of infectious diseases, vaccines, and global health and is a former consultant to the White House. Dr. Bloom enjoyed a distinguished career in bench science as the principal investigator of a laboratory researching the immune response to tuberculosis. He has been extensively involved with the World Health Organization (WHO) for more than 40 years. He was Chair of the Technical and Research Advisory Committee to the Global Programme on

Malaria at WHO and a member of the WHO Advisory Committee on Health Research, as well as chairing the WHO Committees on Leprosy Research and Tuberculosis Research, and the Scientific and Technical Advisory Committee of the United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Dr. Bloom serves on the editorial board of the *Bulletin of the World Health Organization*. Dr. Bloom served on the Ellison Medical Foundation Scientific Advisory Board and the Wellcome Trust Pathogens, Immunology and Population Health Strategy Committee. He was on the Scientific Advisory Board of the Earth Institute at Columbia University and the Advisory Council of the Paul G. Rogers Society for Global Health Research. His past service includes membership on the National Advisory Council of the National Institute for Allergy and Infectious Diseases, the Scientific Advisory Board of the National Center for Infectious Diseases of the Centers for Disease Control and Prevention, and the National Advisory Board of the Fogarty International Center at the National Institutes of Health, as well as the Governing Board of the Institute of Medicine, now known as the National Academy of Medicine. Dr. Bloom was the founding chair of the board of trustees for the International Vaccine Institute in South Korea. He has chaired the Vaccine Advisory Committee of UNAIDS where he played a critical role in the debate surrounding the ethics of AIDS vaccine trials. He was also a member of the U.S. AIDS Research Committee. Dr. Bloom was introduced to the Harvard Chan School as the Dean of Faculty in 1998, and stepped down December 31, 2008. Dr. Bloom now serves as a Harvard University Distinguished Service Professor.

Donald S. Burke is the dean of the Graduate School of Public Health, director of the Center for Vaccine Research, and associate vice chancellor for global health at the University of Pittsburgh. He is also the first occupant of the University of Pittsburgh Medical Center-Jonas Salk Chair in Global Health and a distinguished university professor of health science and policy. He was an intern and resident in medicine at Boston City and Massachusetts General Hospitals and trained as a research fellow in infectious diseases at the Walter Reed Army Medical Center. Dr. Burke has expertise in the prevention and control of infectious diseases of global concern, including HIV/AIDS, influenza, dengue, and emerging infectious diseases. He is an Institute of Medicine (IOM) member (now the National Academy of Medicine) and has served on previous National Research Council and IOM committees, including the Committee on the Special Immunizations Program for Laboratory Personnel Engaged in Research on Countermeasures for Select Agents and the Committee on Assessment of Future Scientific Needs for Live Variola Virus. Dr. Burke received his B.A. from Western Reserve University and his M.D. from Harvard Medical School.

Philip R. Dormitzer is vice president and chief scientific officer for viral vaccines in the Pfizer Vaccine Research and Development Unit. He is a board certified internal medicine physician. After studying anthropology at Harvard College and carrying out a field study of the Efe Pygmies in the Ituri Forest of Zaire, he completed his M.D. and Ph.D. in Cancer Biology at Stanford University. Dr. Dormitzer completed house-staff training in Internal Medicine at Massachusetts General Hospital and a fellowship in the Harvard Combined Infectious Diseases Training Program. As an assistant professor of pediatrics at Harvard Medical School, Dr. Dormitzer led a structural virology laboratory. The Dormitzer group and its collaborators determined the structures of the rotavirus neutralization antigens by nuclear magnetic resonance spectroscopy, X-ray crystallography, and near atomic resolution electron cryomicroscopy. From 2007-2015 Dr. Dormitzer held a series of positions at Novartis Vaccines and Diagnostics, and was global head of research and vice president at a successor company, Novartis Influenza Vaccines. His teams' research and development programs included vaccines targeting influenza, respiratory syncytial virus, cytomegalovirus, HIV, and parvovirus B19. In 2009, he led the research component of the Novartis response to the H1N1 influenza pandemic, supporting the development and licensure of three pandemic influenza vaccines in the most rapid vaccine response in history. In a Biomedical Advanced Research and Development Authority–funded collaboration with the J. Craig Venter Institute and Synthetic Genomics Vaccines, Inc., the Novartis influenza vaccine research team developed a process to synthesize influenza vaccine seed viruses and deployed the technology in response to the H7N9 influenza outbreak in China. The team's other technology platforms included structurally engineered antigens, adjuvants that target toll-like receptors, and self-replicating messenger RNA vaccines.

Baruch Fischhoff is the Howard Heinz University Professor in the departments of Social and Decision Sciences and of Engineering and Public Policy at Carnegie Mellon University, where he heads the Decision Sciences major. A graduate of the Detroit Public Schools, he holds a B.S. in mathematics and psychology from Wayne State University and an M.A. and a Ph.D. in psychology from the Hebrew University of Jerusalem. He is a member of the National Academy of Medicine and is past President of the Society for Judgement and Decision Making and of the Society for Risk Analysis, and recipient of its Distinguished Achievement Award. He was founding chair of the Food and Drug Administration Risk Communication Advisory Committee and recently chaired the National Research Council Committee on Behavioral and Social Science Research to Improve Intelligence Analysis for National Security. Dr. Fischhoff currently co-

chairs the National Research Council Committee on Future Research Goals and Directions for Foundational Science in Cybersecurity and the National Academy of Sciences' Sackler Colloquium on "The Science of Science Communication." He is a former member of the Eugene, Oregon, Commission on the Rights of Women, Department of Homeland Security's Science and Technology Advisory Committee, the World Federation of Scientists Permanent Monitoring Panel on Terrorism, and the Environmental Protection Agency Science Advisory Board, where he chaired the Homeland Security Advisory Committee. He is a fellow of the American Psychological Association, the Association for Psychological Science (previously the American Psychological Society), the Society of Experimental Psychologists, and the Society for Risk Analysis.

Charles N. Haas is the L.D. Betz Chair Professor of Environmental Engineering and Head of the Department of Civil, Architectural, and Environmental Engineering at Drexel University. His broad research interests include drinking water treatment, bioterrorism and risk assessment. Specific research activities include assessment of risks from exposures to deliberately released agents; engineering analysis and optimization of chemical decontamination schemes; microbiological risks associated with pathogens in drinking water, biosolids, and foods; novel kinetic models for disinfection processes and process control; and use of computational fluid dynamics for process modeling. Dr. Haas was co-director of the Center for Advancing Microbial Risk Assessment that is jointly funded by the Department of Homeland Security and the Environmental Protection Agency. He received his Ph.D. from the University of Illinois. He is a past member of the National Academies of Sciences, Engineering, and Medicine's Water Science and Technology Board. He is currently a fellow of multiple societies, including the American Association for the Advancement of Science, American Academy of Microbiology and Society for Risk Analysis.

Michelle M. Mello is Professor of Law at Stanford Law School and Professor of Health Research and Policy at Stanford University School of Medicine. She conducts empirical research into issues at the intersection of law, ethics, and health policy. She is the author of more than 140 articles and book chapters on the medical malpractice system, medical errors and patient safety, public health law, research ethics, the obesity epidemic, pharmaceuticals, and other topics. From 2000-2014, Dr. Mello was a professor at Harvard School of Public Health, where she directed the School's Program in Law and Public Health. In 2013-2014 she completed a Lab Fellowship at Harvard University's Edmond J. Safra Center for Ethics. Dr. Mello teaches courses in torts and public health law. She holds a J.D.

from the Yale Law School, a Ph.D. in Health Policy and Administration from the University of North Carolina at Chapel Hill, and an M.Phil. from Oxford University, where she was a Marshall Scholar. In 2013, she was elected to the National Academy of Medicine, formerly known as the Institute of Medicine.

Sir John Skehel is a graduate of the University College of Wales, Aberystwyth (1962) and gained his Ph.D. from the University of Manchester (1966). He did research at the University of Aberdeen (1965-1968) and was a Helen Hay Whitney Foundation fellow at Duke University and at the Medical Research Council (MRC) National Institute for Medical Research (NIMR) Mill Hill (1968-1971). He was MRC staff scientist at NIMR from 1971 to 2006, Director of the World Health Organization World Influenza Centre from 1975 to 1993, Head of Infections and Immunity from 1985 to 2006 and Director of the NIMR from 1987-2006. He is a visiting scientist in the Division of Virology at The Crick Institute. His research is on the influenza virus hemagglutinin and neuraminidase membrane glycoproteins and the mechanisms of their receptor binding, membrane fusion and enzymic activities. He is a Trustee of the Animal Health Trust. He was elected member of the European Molecular Biology Organization in 1983, fellow of the Royal Society in 1984, member of the Academia Europaea in 1992 and fellow of the Academy of Medical Sciences in 1998 (vice president from 2001-2006) and a foreign associate of the United States National Academy of Sciences in 2014. He was knighted in 1996. He was honorary professor of virology at Glasgow University, Liverpool John Moores University in 2007 and University of Padua (medicine and surgery) in 2010. He is a fellow of the University of Wales and an honorary member of the Society for General Microbiology.

Appendix C

Symposium Agenda

National Academy of Sciences Building
2101 Constitution Avenue NW
Washington, DC 20418
March 10-11, 2016

Thursday, March 10: Overview and Context

8:00 am **Registration**
(coffee and tea will be served)

8:45 **Welcome and Opening Remarks**
Moderator: Harvey Fineberg, Symposium Planning Committee Chair

Ralph J. Cicerone, President, National Academy of Sciences
Margaret Hamburg, Foreign Secretary, National Academy of Medicine
Jo Handelsman, Office of Science and Technology Policy
Carrie Wolinetz, National Institutes of Health

9:15 **Overview of the Draft NSABB Policy Framework and Key Policy Questions**
Moderator: Harvey Fineberg, Symposium Planning Committee Chair

Overview of the NSABB Working Paper
Samuel Stanley, Stony Brook University and NSABB Chair
Harvey Fineberg, Symposium Planning Committee Chair

Open Discussion

10:45 **Break**

11:15 **Informing the Policy Framework: The Risk/Benefit Assessment**
Moderator: Charles Haas, Symposium Planning Committee Member

Lessons from the Risk/Benefit Assessment
Rocco Casagrande, Gryphon Scientific

Comments
Louis (Tony) Cox, Cox Associates
Adam Finkel, University of Pennsylvania
Kara Morgan, Battelle

Open Discussion

12:45 pm **Lunch**
(seating available in the West Court and Members Room - follow signs)

1:45 **The Policy Landscape: United States**
Moderator: Michelle Mello, Symposium Planning Committee Member

Discussants
Gerald Epstein, Department of Homeland Security
Richard Frothingham, Duke University
Lawrence Kerr, Department of Health and Human Services
Philip Potter, St. Jude Children's Research Hospital

Open Discussion

3:15 **Break**

3:45 **The Policy Landscape: International Dimensions of GOF Research**
Moderator: Barry Bloom, Symposium Planning Committee Member

Discussants
Ruxandra Draghia-Akli, European Commission
Keiji Fukuda, World Health Organization

Volker ter Meulen, European Academies Science Advisory Council
Silja Vöneky, University of Freiburg and German Ethics Council

Open Discussion

5:15 **Adjourn**
Reception follows in the Great Hall - all participants welcome

Friday, March 11: Digging Deeper: Key Issues for U.S. Policy Choices

8:30 am **Registration**
(coffee and tea will be served)

9:00 **Informing Policy Design: Insights from the Science of Safety and the Science of Public Consultation**
Moderator: Baruch Fischhoff, Symposium Planning Committee Member

Discussants
Ruthanne Huising, McGill University
Gavin Huntley-Fenner, Huntley-Fenner Advisors
Monica Schoch-Spana, UPMC Center for Health Security

Open Discussion

10:30 **Break**

11:00 **Best Practices to Inform National Policy Design and Implementation: Perspectives of Key Stakeholders in the Biomedical and Public Health Communities**
Moderator: Philip Dormitzer, Symposium Planning Committee Member

Discussants
Michael Callahan, Massachusetts General Hospital and Harvard Medical School
Robert Fisher, U.S. Food and Drug Administration
Jonathan Moreno, University of Pennsylvania
Ethan Settembre, Seqirus

Open Discussion

12:30 pm **Lunch**
(seating available in the West Court and Members Room - follow signs)

1:30 **International Governance: Opportunities for Harmonizing GOF Research Policy and Practice**
Moderator: Ronald Atlas, Symposium Planning Committee Member

Discussants
Nisreen AL-Hmoud, Royal Scientific Society of Jordan
George F. Gao, Chinese Academy of Sciences and China CDC
Gabriel Leung, University of Hong Kong
Michael Selgelid, Monash University
Herawati Sudoyo, Indonesian Academy of Sciences and Eijkman Institute for Molecular Biology[1]

Open Discussion

3:00 **Break**

3:30 **Summing Up**
Moderator: Harvey Fineberg, Symposium Planning Committee Chair

Summary Remarks
Brief remarks from the moderators of the plenary sessions to summarize what emerged from the discussions during the symposium to inform the NSABB's recommendations and the U.S. government's policy choices.

Open Discussion

Concluding Remarks
Harvey Fineberg, Symposium Planning Committee Chair

5:00 **Adjourn**

[1] Dr. Sudoyo was unable to take part in the symposium

Appendix D

Speaker and Panelist Biographies

Nisreen AL-Hmoud obtained a Ph.D. in microbiology from Abertay University, Dundee, Scotland in 2002. In 2003, she joined the Royal Scientific Society (RSS) of Jordan as a researcher, and since 2009, she has been leading the group of Biosafety at RSS. Dr. AL-Hmoud is a member of the National Biosafety Committee and the National Committee for Science and Technology Ethics in Jordan. She also served as president of the Biosafety and Biosecurity International Consortium (BBIC) steering committee between May 2010 and July 2012. In October 2015, Dr. AL-Hmoud was appointed as director of the Centre for Excellence in Biosafety, Biosecurity and Biotechnology at RSS. Dr. AL-Hmoud started her teaching career in October 2006 as a visiting lecturer of medical microbiology at the department of Biology, Faculty of Science, at the University of Jordan. In February 2008, she joined Princess Sumaya University for Technology (PSUT) as an assistant professor, and later on as a department head and coordinator for the master's program of Environmental Technology and Management. She is also a lecturer at the Health and Community Development Program of Jordan and the School for International Training Study Abroad Program.

Michael Callahan is a physical scientist boarded in both internal medicine and infectious diseases and is a Diplomat of Mass Casualty Care and Tropical Medicine and Hygiene (UK). Dr. Callahan received his M.S. in International Public Health and his M.D. from the University of Alabama School of Medicine, where he was the 19th Tinsley Harrison Scholar

and received three academic and research awards in his graduate and medical training. His biodefense clinical research is focused on vaccine defeat, immune evade and multidrug-resistant organisms, and on best practices for highly dangerous pathogen infections in Africa where he prospectively enrolls cutaneous anthrax in Nigeria; and monkey pox, Ebola and Marburg in the Democratic Republic of the Congo and Angola. In 2002, he was appointed clinical director for Cooperative Threat Reduction programs at six former Soviet Union (ex) Biological Weapons Institutes (VECTOR, State Research Center for Applied Microbiology, Kirov, Bersk, RCMDT, Highly Pure, and RIHOP), which included redirecting of unanticipated dual use and gain-of-function programs. From 2005 to 2012, Dr. Callahan led the Defense Advanced Research Projects Agency (DARPA) biodefense therapeutics portfolio, which he expanded from $61 million to $260 million per annum in 2011, involving eight programs that generated nine investigational new drugs (INDs) and three new drug applications with products in market. While at DARPA he launched the Department of Defense Icon program Accelerated Manufacture of Pharmaceuticals (AMP), for which he received the 2010 DARPA Achievement Award, and which generated emergency use good manufacturing practice pH1N1 vaccines, and Nicotinia-expressed monoclonals such as ZMapp. Also while at DARPA, he launched Prophecy, the international physician Early Alert network, which delivers 24/7 emergency consultation, reagents and therapeutics for catastrophic (mass-casualty or HDP) infectious disease outbreaks, severe acute respiratory syndrome Hong Kong and H7N9 Nanjing. His drugs in market include Ambisome (Gilead), which has generated $6 billion since approval, cPG100, and four private-sector INDs involving novel anti-infectives, cytotherapeutics, or host-based antivirals. Dr. Callahan is president of United Therapeutics (UTHR) Division of Cell Therapeutics, and maintains faculty appointments at Massachusetts General Hospital/Harvard Medical School and King Chulalongkom Medical University in Bangkok. Dr. Callahan continues his federal service as infectious disease and biosafety SME to the Academies, the National Security Council, BSEG, the Office of Net Assessment, National Institute of Allergy and Infectious Diseases, MITRE, American Society of Microbiology, Infectious Disease Society of America, and the American Society of Tropical Medicine and Hygiene.

Rocco Casagrande is the Managing Director of Gryphon Scientific, LLC. His projects at Gryphon Scientific focus on bringing rigorous scientific analysis to problems of homeland defense. For the past dozen years, Dr. Casagrande has led more than 50 projects to evaluate and improve U.S. preparedness efforts for a chemical, biological, radiological, and nuclear attack or emerging infectious disease event and to support a

better understanding of the threat. Dr. Casagrande also served as the principal investigator of several projects supporting the U.S. government's stance on emerging biotechnologies, including the guidance to the synthetic DNA industry and its moratorium on funding research involving engineered influenza viruses. From December 2002 to March 2003, Dr. Casagrande served as an United Nations Monitoring, Verification and Inspection Commission (UNMOVIC) biological weapons inspector in Iraq where he acted as the chief of the United Nations biological analysis laboratory. Prior to working for UNMOVIC, Dr. Casagrande worked in private industry as an inventor in a nano/biotechnology company. Dr. Casagrande holds a B.A. in chemistry and biology from Cornell University, where he graduated magna cum laude, and a Ph.D. in biology from the Massachusetts Institute of Technology.

Ralph J. Cicerone is the president of the National Academy of Sciences. His research in atmospheric chemistry, climate change, and energy has involved him in shaping science and environmental policy at the highest levels nationally and internationally. Dr. Cicerone was educated at the Massachusetts Institute of Technology (B.S. in electrical engineering) and the University of Illinois at Champaign-Urbana (M.S., Ph.D. in electrical engineering, with a minor in physics). In his early career, he was a research scientist and held faculty positions in electrical and computer engineering at the University of Michigan. The Ralph J. Cicerone Distinguished University Professorship of Atmospheric Science was established there in his honor in 2007. In 1978, he joined the Scripps Institution of Oceanography at the University of California, San Diego, as a research chemist. From 1980 to 1989, he was a senior scientist and director of the Atmospheric Chemistry Division at the National Center for Atmospheric Research in Boulder, Colorado. In 1989, he joined the University of California, Irvine, where he was founding chair of the Department of Earth System Science and was appointed the Daniel G. Aldrich Professor of Earth System Science. As dean of the School of Physical Sciences from 1994 to 1998, he recruited outstanding faculty and strengthened the school's curriculum and outreach programs. Immediately prior to his election as Academy president, Dr. Cicerone served as Chancellor of University of California, Irvine, from 1998 to 2005, a period marked by a rapid rise in the academic capabilities of the campus. His research has focused on atmospheric chemistry, the radiative forcing of climate change due to trace gases, and the sources of atmospheric methane, nitrous oxide, and methyl halide gases.

Louis "Tony" Cox is president of Cox Associates, a Denver-based applied research company specializing in quantitative health risk analysis, casual modeling, advanced analytics, and operations research. Since 1986, Cox

Associates' mathematicians and scientists have applied computer simulation, biomathematical models, biostatistical and epidemiological risk analyses, casual data mining, machine learning, biomathematical modeling and bioinformatics, operations research, and artificial intelligence models to measurably improve health and engineering risk assessment and decision making for public and private sector clients. In 2006, Cox Associates was inducted into the Edelman Academy of the Institute for Operations Research and Management Science (INFORMS), recognizing outstanding real-world achievements in the practice of operations research and the management sciences. In 2012, Dr. Cox was inducted into the National Academy of Engineering (NAE) "for applications of operations research and risk analysis to significant national problems." He is a member of the Academies Board on Mathematical Sciences and their Applications (BMSA) and a member of the Academies Standing Committee on the Use of Public Health Data in Food Safety and Inspection Service Food Safety Programs. Dr. Cox holds a Ph.D. in Risk Analysis (1986) and an S.M. in Operations Research (1985), both from the Massachusetts Institute of Technology; an A.B. from Harvard University (1978); and is a graduate of the Stanford Executive Program (1993). He is honorary full professor of Mathematics at the University of Colorado, Denver, where he has lectured on risk analysis, biomathematics, health risk modeling, computational statistics and causality; is on the Faculties of the Center for Computational Mathematics and the Center for Computational Biology; and is clinical professor of Biostatistics and Informatics at the University of Colorado Health Sciences Center. Dr. Cox is editor-in-chief of *Risk Analysis: An International Journal*, is area editor for Real World Application for the *Journal of Heuristics*, and is on the Editorial Board of the *International Journal of Operations Research and Information Systems*. He is an Edelman Laureate of INFORMS, a member of the American Statistical Association (ASA), and a fellow of the Society for Risk Analysis (SRA).

Ruxandra Draghia-Akli joined DG Research and Innovation of the European Commission as health director in 2009. In her position, Dr. Draghia-Akli is constantly seeking to deepen the reach, the breadth, and the depth of Europe's excellence in health research and innovation (R&I). Before joining the European Commission, Dr. Draghia-Akli served as vice president of Research at VGX Pharmaceuticals (now Inovio) and VGX Animal Health. She received an M.D. from Carol Davilla Medical School and a Ph.D. in human genetics from the Romanian Academy of Medical Sciences. She also completed a doctoral fellowship at the University of Rene Descartes in Paris and post-doctoral training at Baylor College of Medicine and served as faculty at Baylor. In 2012, she became an honorary member of the Romanian Academy of Medical Sciences.

Gerald Epstein is a fellow of the American Physical Society and the American Association for the Advancement of Science. He serves on the editorial board for the journal *Biosecurity and Bioterrorism* and has served on the Biological Threats Panel of the National Academy of Sciences' Committee on International Security and Arms Control and the Biological Sciences Experts Group for the Office of the Director of National Intelligence. He also served on the Committee on Science, Security, and Prosperity, which produced the report *Beyond Fortress America: National Security Controls on Science and Technology in a Globalized World*. He received B.S. degrees in physics and electrical engineering from the Massachusetts Institute of Technology and a Ph.D. in physics from the University of California, Berkeley.

Adam Finkel is currently executive director of the Penn Program on Regulation at the University of Pennsylvania, where he is also a senior fellow at the Penn Law School, and is clinical professor of Environmental Health Sciences at the University of Michigan School of Public Health. From 2004 to 2007, he was a visiting professor of Public and International Affairs at the Woodrow Wilson School at Princeton University. From 2000 to 2003, Dr. Finkel was regional administrator for the U.S. Occupational Safety and Health Administration (OSHA) in Denver, Colorado, responsible for regulatory enforcement, compliance assistance, and outreach activities in the six-state Rocky Mountain region (Region VIII). From 1995 to 2000, he was Director of Health Standards Programs at OSHA headquarters, and was responsible for promulgating and evaluating regulations to protect the nation's workers from chemical, radiological, and biological hazards. Dr. Finkel holds an Sc.D. in environmental health sciences from the Harvard School of Public Health, an M.A. in public policy from Harvard's John F. Kennedy School of Government, an A.B. in biology from Harvard College, and is a certified industrial hygienist. Dr. Finkel has pioneered methodological improvements in human health risk assessment and cost–benefit analysis for the past 25 years, primarily in the areas of quantitative uncertainty analysis, accounting for interindividual variability in susceptibility, and designing regulatory processes to maximize stakeholder input and shed light on economic impacts. He is co-author of four books, including the 2014 volume *Does Regulation Kill Jobs?* In 2006, he received the David P. Rall Award for Advocacy in Public Health from the American Public Health Association for "a career in advancing science in the service of public health protection." In 2013, he received the Alumni Leadership in Public Health Practice Award from the Harvard School of Public Health.

Robert Fisher is director, Regulatory Science for the Food and Drug Administration's (FDA's) Office of Counterterrorism and Emerging

Threats (OCET) and the Medical Countermeasures Initiative (MCMi). He leads the MCMi Regulatory Science Program, oversees intra- and extramural research programs, and works with FDA Centers, PHEMCE stakeholders, and other U.S. and international partners on medical countermeasure-related regulatory science issues. Dr. Fisher joined FDA's Center for Biologics Research and Review (CBER) as a staff fellow in 2006, and served as a staff scientist from 2013-2015. During his tenure at CBER, he provided scientific leadership for regulatory review of chemical, biological, radiological, and nuclear (CBRN) medical countermeasures. He maintained an active research interest in several medical countermeasure related fields, including the modeling of complications related to vaccinia live-virus vaccines and investigating methods for improved characterization of botulism and anthrax antitoxin products. Dr. Fisher received his undergraduate degree in biology from the University of North Carolina at Pembroke and a Ph.D. in toxicology from the University of North Carolina at Chapel Hill. He studied filovirus and poxvirus pathogenesis under a National Research Council Research Associateship at the U.S. Army Medical Research Institute of Infectious Diseases and holds a certificate in Biohazardous Threat Agents and Emerging Infectious Diseases from Georgetown University.

Richard Frothingham is an associate professor of Medicine at Duke University Medical Center. He received his B.S. from the Massachusetts Institute of Technology and his M.D. from Duke. He completed clinical training programs in Medicine, Pediatrics, and Infectious Diseases and maintains board certification in Infectious Diseases. He is also a Certified Biological Safety Professional. Dr. Frothingham directs the National Institute of Allergy and Infectious Diseases Regional Biocontainment Laboratory at Duke University. This laboratory was built to support research to develop drugs, diagnostics, and vaccines for emerging infections and biological threats. The Frothingham lab studies host responses to tuberculosis with the goal of developing better vaccines and treatments. Dr. Frothingham also provides clinical care to persons with HIV infection. Dr. Frothingham serves as co-chair of the Duke Institutional Biosafety Committee (IBC). The Duke IBC has reviewed and managed biological research with the potential for dual use since 2005.

Keiji Fukuda is special representative for antimicrobial resistance for the director-general at the World Health Organization (WHO). He previously served as the assistant director-general for health security, the special adviser on pandemic influenza to the director-general, and director of the Global Influenza Programme. Before joining WHO, Dr. Fukuda served as the chief of the Epidemiology Unit, Influenza Branch, Centers for Disease

Control and Prevention (CDC) in the United States. He has extensive global and national public health experience with health security and emerging infectious diseases, including field investigations and research, capacity building and preparedness, communications, surveillance, and with international governance and frameworks such as the International Health Regulations, the Pandemic Influenza Preparedness Framework and the Codex Alimentarius. He is currently focusing on shaping the global approach to antimicrobial resistance. Dr. Fukuda is a physician and epidemiologist and received his B.A. from Oberlin College, his M.D. from the University of Vermont, his M.P.H. from the University of California, Berkeley, and additional training in epidemiology at CDC.

George Gao obtained his Ph.D. (D.Phil.) degree in 1995 from Oxford University, United Kingdom. He was selected by the Chinese Academy of Sciences "Hundred Talents" program in 2004, and received the National Natural Science Foundation of China (NSFC) Distinguished Young Scholar title in 2005. He is the chief scientist of two consecutive projects on the mechanism of interspecies transmission of viral pathogens and a leading principal investigator of the NSFC Innovative Research Group. He is also a member of the steering committee for the International Consortium of Anti-Virals (ICAV), and a visiting professor at Oxford University. He was awarded the World Academy of Sciences (TWAS) prize in Medical Sciences in 2012 and the Nikkei Asia Prize in 2014. Dr. Gao is a member of the Chinese Academy of Sciences, a fellow of TWAS, a fellow of the American Academy of Microbiology, and the director and professor in the Chinese Academy of Sciences Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology. He is also the vice president of the Beijing Institutes of Life Science, Chinese Academy of Sciences, Deputy Director-General of the Chinese Center for Disease Control and Prevention, and dean of the UCAS Cunji College of Medicine.

Margaret Hamburg earned her B.A. from Harvard College, her M.D. from Harvard Medical School, and completed her residency at what is now New York Presbyterian Hospital-Weill Cornell Medical Center. She conducted neuroscience research at Rockefeller University in New York and at the National Institute of Mental Health, and later focused on HIV/AIDS research and policy as assistant director of the National Institute of Allergy and Infectious Diseases. In 1991, after just 1 year in the New York City Department of Health, Dr. Hamburg was named its commissioner. During her 6-year tenure, she implemented rigorous public health initiatives that tackled the city's most pressing crises head-on—including improved services for women and children, a needle-exchange program to combat HIV transmission, and the nation's first public health bioterrorism

defense program. The most celebrated achievement during her leadership was her aggressive approach to the city's tuberculosis epidemic, which led to an 86 percent decline in drug-resistant tuberculosis in just 5 years. In 1997, 3 years after she was elected one of the youngest-ever members of the Institute of Medicine, President Bill Clinton named Dr. Hamburg assistant secretary for planning and evaluation in the Department of Health and Human Services, where she served until the end of the Clinton Administration. She then became founding vice president for biological programs at the Nuclear Threat Initiative, a foundation dedicated to reducing the threat to public safety from nuclear, chemical, and biological weapons. President Barack Obama nominated Dr. Hamburg for the post of the Food and Drug Administration commissioner on March 14, 2009. Dr. Hamburg is a member of the National Academy of Medicine and currently serves as its foreign secretary.

Jo Handelsman is the associate director for science at the White House Office of Science and Technology Policy (OSTP), appointed by President Obama and confirmed by the Senate in June of 2014. Dr. Handelsman helps to advise President Obama on the implications of science for the nation, ways in which science can inform U.S. policy, and on federal efforts in support of scientific research. Prior to joining OSTP, Dr. Handelsman was the Howard Hughes Medical Institute Professor and Frederick Phineas Rose Professor in the Department of Molecular, Cellular and Developmental Biology at Yale University. She previously served on the University of Wisconsin, Madison, faculty as a professor in plant pathology from 1985 to 2009 and as professor and chair of the Department of Bacteriology from 2007 to 2009. In 2013, she served as president of the American Society for Microbiology. Dr. Handelsman is an expert in communication among bacteria that associate with soil, plants, and insects and helped pioneer the field of metagenomics, bridging agricultural and medical sciences. Dr. Handelsman is also recognized for her research on science education and women and minorities in science, and received the Presidential Award for Excellence in Science Mentoring in 2011. Dr. Handelsman also co-chaired the PCAST working group that developed the 2012 report, "Engage to Excel," which contained recommendations to the president to strengthen STEM (science, technology, engineering, and mathematics) education to meet the workforce needs of the next decade in the United States. Dr. Handelsman received a B.S. from Cornell University and a Ph.D. in Molecular Biology from the University of Wisconsin, Madison.

Ruthanne Huising is an ethnographer of work and organizations. She studies how organizations respond to external pressures to change and the implications of these changes for professional control and expertise.

Across various projects she has observed how organizations accommodate regulatory change (Canada's Human Pathogens and Toxins Act), auditing fads (Environment, Health & Safety Management Systems), and efficiency efforts (Ontario's perioperative coaching program), and the complex responses of scientists, biosafety officers, health physicists, surgeons, nurses, and administrators. Ruthanne is an associate professor in the faculty of management at McGill University. She received her Ph.D. from the Sloan School of Management at the Massachusetts Institute of Technology.

Gavin Huntley-Fenner is an independent human factors consultant. His consulting and research interests are focused on the contribution of risk perception and reasoning to warnings effectiveness. Prior to focusing full-time as a human factors consultant, Dr. Huntley-Fenner was a business consultant at McKinsey & Company. He began his professional career as an assistant professor at the University of California, Irvine, after earning his Ph.D. in Brain and Cognitive Sciences from the Massachusetts Institute of Technology. From 2010-2014, he served as a member of the Food and Drug Administration's Risk Communication Advisory Committee.

Lawrence Kerr is the director of pandemics and emerging threats within the Office of Global Affairs at the Department of Health and Human Services (HHS). Dr. Kerr leads and manages the office, overseeing a broad policy portfolio including the global health security agenda implementation, pandemics and emerging threats, antimicrobial resistance, security policy issues (biosafety and biosecurity, biothreat prevention [Biological Weapons and Toxins Convention, United Nations Security Council 1540, Global Partnership against the Spread of Materials and Weapons of Mass Destruction]), and dual use research of concern. Prior to joining HHS in December 2015, Dr. Kerr served as the director for medical preparedness policy in the Resilience Directorate at the White House National Security Council Staff as the principal staff member responsible for coordinating policy regarding public health and medical resilience for biological events, whether the results of naturally emerging disease or deliberate release including his role on the Ebola Task Force. He previously served as the senior bio advisor to the director of the National Counterproliferation Center (NCPC) within the Office of the Director of National Intelligence. Dr. Kerr advised the senior leadership on strategic plans to prevent and counter the spread of biological weapons of mass destruction. Before joining NCPC in April 2006, he was director for biodefense policy with the White House Homeland Security Council in the Executive Office of the President. He served as assistant director for homeland security for the Office of Science and Technology Policy (OSTP) and as director

of bioterrorism, research and development for the Office of Homeland Security in the Executive Office of the President. Dr. Kerr joined the Life Sciences division of OSTP in January 2001, where he came from his position at the National Institute of Allergy and Infectious Diseases at the National Institutes of Health. He holds a B.S. in Biology and Art History and a Ph.D. in Cell Biology, both from Vanderbilt University.

Gabriel Leung is dean of medicine and chair professor of public health medicine at The University of Hong Kong. Previously he was Hong Kong's first under secretary for food and health, then director of the chief executive's office in government. Dr. Leung is one of Asia's leading epidemiologists, having authored more than 400 scholarly papers and edited numerous leading journals. He directs the university's World Health Organization Collaborating Centre for Infectious Disease Epidemiology and Control. His research defined the epidemiology of two novel viral epidemics, namely severe acute respiratory syndrome coronavirus in 2003 and influenza A (H7N9) in 2013. While in government, he led Hong Kong's policy response against the 2009 influenza A (H1N1) pandemic.

Jonathan Moreno is 1 of 16 Penn Integrates Knowledge university professors at the University of Pennsylvania, holding the David and Lyn Silfen chair. He is also professor of medical ethics and health policy, of history and sociology of science, and of philosophy. Dr. Moreno is a senior fellow at the Center for American Progress in Washington, DC. In 2008-2009 he served as a member of President Obama's transition team. His work has been cited by Al Gore and was used in the development of the screenplay for *The Bourne Legacy*. His online neuroethics course drew more than 36,000 registrants in 2013. Dr. Moreno's writings have been translated into Chinese, German, Japanese, and Portugese. The *American Journal of Bioethics* has called him "the quietly most interesting bioethicist of our time." Dr. Moreno is an elected member of the National Academy of Medicine. He has served as a senior staff member for three presidential advisory commissions, including the current bioethics commission under President Obama, and has given invited testimony for both houses of Congress. Dr. Moreno is the U.S. member of the United Nations Educational, Scientific and Cultural Organization International Bioethics committee. Dr. Moreno received his Ph.D. in philosophy from Washington University in St. Louis, was an Andrew W. Mellon postdoctoral fellow, holds an honorary doctorate from Hofstra University, and is a recipient of the Benjamin Rush Medal from the College of William and Mary Law School and the Dr. Jean Mayer Award for Global Citizenship from Tufts University.

Kara Morgan has 16 years of experience in risk analysis and decision analysis. She earned her B.S. in Mathematics from Michigan State University, her M.S. in Environmental Science from Indiana University, and her Ph.D. in Engineering and Public Policy from Carnegie Mellon University. After earning her Ph.D., she worked for 4 years at Research Triangle Institute, supporting the Environmental Protection Agency with the use of data-based decision making methods. Then, she spent 10 years at the Food and Drug Administration, working to support the development and implementation of risk-based decision-making tools and to implement strategic program planning for improving the achievement of outcomes. She is currently a research leader at Battelle Memorial Institute in the Health and Analytics sector. In that role, she works with clients to improve their use of data to inform decision making, supports knowledge management tasks related to quality measures for health care improvement, and works with clients to assess the outcomes their programs are achieving. She is also an adjunct professor at the Ohio State University's Glenn College of Public Affairs, where she teaches courses on risk and decision analysis. Dr. Morgan's professional focus has been on developing tools and methods for supporting effective data-driven risk management decisions. Her areas of emphasis include performance measurement, strategic planning, program evaluations, knowledge management, risk and decision analysis, and application of these tools and methods to improve decision making and improve outcomes.

Philip Potter obtained his Ph.D. in molecular carcinogenesis at the Paterson Institute for Cancer Research in Manchester, United Kingdom, and moved to St. Jude Children's Research Hospital in Memphis, Tennessee, shortly thereafter. His laboratory has worked for many years on the modulation of the response of tumor cells to chemotherapy, using both small molecule and molecular approaches. The latter has principally involved the use of adenovirus to deliver agents, such as ribozymes and drug metabolizing enzymes to cells, both in vitro and in vivo. Consequently, he has expertise in the design and construction of viral vectors and their practical use in the laboratory. Dr. Potter has more than 11 years of experience serving on the St. Jude Institutional Biosafety Committee, including as vice chairman and chairman. He is currently the vice chair of the IBC, and the chairman of the dual use research of concern subcommittee for the institution.

Monica Schoch-Spana, a medical anthropologist, is a senior associate with the University of Pittsburgh Medical Center (UPMC) Center for Health Security and a faculty member with the School of Medicine at the University of Pittsburgh and the Department of Anthropology at Texas State University. Dr. Schoch-Spana is a leading social science researcher in

public health emergency preparedness. Her studies have been influential in debunking myths about mass behaviors in the context of bioterrorism and other health crises and in reframing the management of catastrophic health events to include social, ethical-moral, and governance dimensions. National advisory roles include serving on the Homeland Security Subcommittee of the Board of Scientific Counselors for the Environmental Protection Agency, the Resilient America Roundtable of the National Academy of Sciences, and the National Research Council Committee on Increasing National Resilience to Hazards and Disasters. Dr. Schoch-Spana has chaired national working groups to produce peer-reviewed, evidence-based consensus guidance for authorities on how to partner with citizens and civil society in relation to bioterrorism response, influenza pandemic planning, and nuclear incident preparedness, and she has organized three national meetings on how to strengthen community resilience to extreme health events. Her current research projects focus on local health department capacity for community engagement, communication dilemmas concerning medical countermeasures, and public participation in the development of policies for allocating scarce medical resources in a disaster. In 2003, Dr. Schoch-Spana helped establish the UPMC Center for Health Security. Prior to that, she worked at the Johns Hopkins University Center for Civilian Biodefense Strategies starting in 1998. She received her Ph.D. in cultural anthropology from Johns Hopkins University and a B.A. from Bryn Mawr College.

Ethan Settembre is vice president and head of research for Seqirus. He holds a Ph.D. in biochemistry from Cornell University and completed his postdoctoral training in Structural Virology at Harvard Medical School. He then joined Novartis Vaccines & Diagnostics in 2008 where he held several key positions in research developing vaccines against multiple viral targets, including influenza. Currently, he heads the Seqirus Research group focused on influenza vaccine development.

Michael Selgelid is director of the Centre for Human Bioethics, and the World Health Organization (WHO) Collaborating Centre for Bioethics therein, at Monash University in Melbourne, Australia. He is a member of the board of directors of the International Association of Bioethics and serves on the Ethics Review Board of Médecins Sans Frontières. His main research focus is public health ethics with emphasis on ethical issues associated with infectious disease. He edits a book series in Public Health Ethics Analysis for Springer and a book series in Practical Ethics and Public Policy for ANU Press. He is co-editor of *Monash Bioethics Review* and an associate editor of the *Journal of Medical Ethics*. Dr. Selgelid earned a B.S. in

Biomedical Engineering from Duke University, and a Ph.D. in Philosophy from University of California, San Diego.

Samuel L. Stanley, Jr. was appointed as the fifth president of Stony Brook University in May 2009. Since that time he has presided over a tremendous growth of the university, through the implementation of a faculty hiring program that has brought 200 net new faculty to Stony Brook, a 5-fold increase in endowed professorships, the largest number of applicants and most accomplished classes in the school's history, and record fundraising totals, including one of the largest gifts ever to a public university. Before becoming president of Stony Brook University, Dr. Stanley served as vice chancellor for research at Washington University in St. Louis, where he had a distinguished career as a biomedical researcher with a focus on host defense against emerging pathogens. Dr. Stanley currently serves as the chair of the National Science Advisory Board for Biosecurity (NSABB), is a member of the National Security Higher Education Advisory Board (NSHEAB), is the chair of Brookhaven Science Associates (BSA), which manages Brookhaven National Laboratory, is a member the board of directors of Cold Spring Harbor Laboratory, and is a member of the board of directors of the Research Foundation, State University of New York.

Volker ter Meulen qualified as an M.D. in 1960. He received his post-doctoral training in virology in the United States, at the Children's Hospital of Philadelphia. On returning to Germany in 1996, he specialized in pediatrics and was subsequently visiting scientist at the Wistar Institute for Anatomy and Biology in Philadelphia and at the Viral and Rickettsial Disease Laboratory in Berkeley, from 1969-1970. In 1975 he became a full professor and chairman of the Institute of Virology and Immunobiology at the University of Würzburg. He retired in 2002, having twice been elected dean of the faculty of medicine at Würzburg University. During his research career, Dr. ter Meulen worked on molecular and pathogenic aspects of viral infections in man and animals, in particular on infections of the central nervous system. Internationally, Dr. ter Meulen has served on a number of committees of organisations and scientific societies/unions in the area of virology and infectious diseases, covering a broad spectrum of important issues connected to human and animal pathogens. From 2003-2010, Dr. ter Meulen was president of the German Academy of Sciences Leopoldina. From 2007-2010, he was president of the European Academies Science Advisory Council (EASAC), the association of the National Science Academies of the European Union, which is the IAP associated regional network for Europe. He was elected IAP Co-Chair in February 2013.

Silja Vöneky is co-director of the Institute for Public Law and is a professor of public international law, comparative law, and ethics of law at the University of Freiburg (Germany). Her areas of focus include international law, international humanitarian law, international environmental law, the law of the sea, international protection of human rights, the relation of ethics and law, and especially questions on how to regulate existential risks (biosecurity law and democratic legitimacy.) Since 2001, Professor Vöneky has served as the legal adviser to the German Federal Foreign Office, German Federal Ministry of Research, German Federal Ministry of the Environment, and the Alfred Wegener Institute for Scientific Marine and Polar Research. Since 2012, she has been a member of the German Ethics Council, appointed on the proposal of the federal government, and was the head of the Working Group on Biosecurity of the German Ethics Council.

Carrie Wolinetz is associate director for science policy and director of the Office of Science Policy (OSP) at the National Institutes of Health (NIH). As leader of OSP, she advises the NIH director on science policy matters of significance to the agency, the research community, and the public on a wide range of issues, including human subjects protections, biosecurity, biosafety, genomic data sharing, regenerative medicine, the organization and management of NIH, and the outputs and values of NIH-funded research. Prior to joining NIH, Dr. Wolinetz worked on biomedical research policy issues as the deputy director for federal affairs at the Association of American Universities (AAU) and the director of scientific affairs and public relations at the Federation of American Societies for Experimental Biology (FASEB). She also served as the president of United for Medical Research, a leading NIH advocacy coalition. Outside of NIH, Dr. Wolinetz teaches as an adjunct assistant professor at Georgetown University in the School of Foreign Service's program on Science, Technology & International Affairs. She has a B.S. in animal science from Cornell University, and she received her Ph.D. in animal science from Pennsylvania State University, where her area of research was reproductive physiology.

Appendix E

List of Attendees

Nisreen AL-Hmoud
Royal Scientific Society of Jordan

Abdulaziz Alagaili
King Saud University

Lida Anestidou
National Academies of Sciences, Engineering, and Medicine

Ronald Atlas
University of L ouisville

Rachel Bartholomew
Pacific Northwest National Laboratory

Kavita Berger
Gryphon Scientific

Kenneth Berns
University of Florida

Lizbet Boroughs
Association of American Universities

Donald Burke
University of Pittsburgh

Michael Callahan
Massachusetts General Hospital
Harvard Medical School

Elizabeth Cantwell
Lawrence Livermore National Laboratory

Sarah Carter
J. Craig Venter Institute

Rocco Casagrande
Gryphon Scientific

Ralph Cicerone
National Academy of Sciences

Louis "Tony" Cox
Cox Associates

Bruce Crise
Institutional Biosafety Committee Consultant

Genevieve Croft
Association of Public and Land-grant Universities

Patricia Delarosa
National Institute of Allergy and Infectious Diseases

Diane DiEuliis
National Defense University

Philip Dormitzer
Pfizer Vaccine Research and Development

Ruxandra Draghia-Akli
European Commission

David Drew
Woodrow Wilson Center

Leo Einck
EpiVax, Inc.

Gerald Epstein
Department of Homeland Security

Nicholas Evans
University of Pennsylvania

Harvey Fineberg
Gordon and Betty Moore Foundation

Adam Finkel
University of Pennsylvania

Andi and Baruch Fischhoff
Carnegie Mellon University

Robert Fisher
Food and Drug Administration

J. Patrick Fitch
National Biodefense Analysis and Countermeasures Center

Meg Flanagan
Department of State

Greg Frank
Infectious Diseases Society of America

Richard Frothingham
Duke University

Keiji Fukuda
World Health Organization

George F. Gao
Chinese Academy of Sciences
Chinese Center for Disease Control and Prevention

Liz Geltman
CUNY School of Public Health

Daniel Feakes
Biological Weapons Convention

Ashley Grant
Government Accountability Office

Gigi Gronvall
University of Pittsburgh Medical Center Center for Health Security

Charles Haas
Drexel University

Margaret Hamburg
National Academy of Medicine

Marie-Louise Hammarskjöld
University of Virginia

Jo Handelsman
Office of Science and Technology Policy
The White House

Chris Hanson
National Institute of Allergy and Infectious Diseases

Brit Hart
Association of Public Health Laboratories

Jack Herrmann
National Academies of Sciences, Engineering, and Medicine

Rona Hirschberg
Rona Hirschberg Consulting LLC

Andrea Hodges
National Academies of Sciences, Engineering, and Medicine

Ruthanne Huising
McGill University

Gavin Huntley-Fenner
Huntley-Fenner Advisors

Jo L. Husbands
National Academies of Sciences, Engineering, and Medicine

Tom Inglesby
University of Pittsburgh Medical Center Center for Health Security

Katherine Jones
Sandia National Laboratories

Joseph Kanabrocki
University of Chicago

Luba Katz
Abt Association

Lawrence Kerr
Department of Health and Human Services

Andy Kilianski
Edgewood Chemical Biological Center

Tamika Knight
Department of Homeland Security

Gregory Koblentz
George Mason University

Margaret Kosal
Georgia Tech

Amy Krafft
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Viktoriya Krakovna
Future of Life Institute

Jan Leach
Colorado State University

James LeDuc
University of Texas Medical Branch

Betty Lee
Department of Commerce

Gabriel Leung
The University of Hong Kong

Rachel Levinson
Arizona State University

Carol Linden
Office of the Chief Scientist
Food and Drug Administration

Marc Lipsitch
Harvard T.H. Chan School of Public Health

Daniel Lucey
Georgetown University

Francis Macrina
Virginia Commonwealth University

Joanne Manrique
Center for Global Health and Diplomacy

Monique Mansoura
Seqirus

Brendan McGovern
National Academies of Sciences, Engineering, and Medicine

Michelle Mello
Stanford University School of Medicine

Carissa Meyer
Gryphon Scientific

Robin Miller
National Academies of Sciences, Engineering, and Medicine

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Biosecure Ltd.

Allison Mistry
Gryphon Scientific

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Renaissance Today

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Kara Morgan
Battelle

Rebecca Moritz
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Atsuko Polzin
Seqirus

Philip Potter
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Philip Price
Wellcome Trust

Kevin Ramkissoon
National Institutes of Health

Catherine Rhodes
University of Cambridge

Ryan Ritterson
Gryphon Scientific

Ben Rusek
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Frederik Schagen
Netherlands Commission on Genetic Modification

Monica Schoch-Spana
University of Pittsburgh Medical Center Center for Health Security

Michael Selgelid
Monash University

Sarath Seneviratne
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Susan Wolf
University of Minnesota

Carrie Wolinetz
National Institutes of Health

Scott Wollek
National Academies of Sciences, Engineering, and Medicine

David Woodland
Keystone Symposia


Risk and Benefit Analysis of Gain of Function Research
Final Report—April 2016
GRYPHON SCIENTIFIC

Risk and Benefit Analysis of Gain of Function Research

This work was conducted under NIH Contract# HHSN263201500002C with Gryphon Scientific from March 20, 2015 to December 15, 2015. Revisions were made until April 2016.

Susan Apter was the NIH contracting officer and Kelly Fennington was her representative

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Project Team:

Gryphon Scientific, Prime Contractor, Rocco Casagrande, Principal Investigator
Signature Science, Subcontractor
Abt Associates, Subcontractor

- **Chapter 1, Executive Summary:** Corey Meyer, Kavita Berger and Rocco Casagrande
- **Chapter 2, Introduction:** Rocco Casagrande
- **Chapter 3, Overview of Methodology:** Corey Meyer, Kavita Berger, Robert Stephan and Rocco Casagrande
- **Chapter 4, Background:** Erin Lauer, Mikaela Finnegan, Corey Meyer and Rocco Casagrande
- **Chapter 5, Historical Context:** Erin Lauer and Rocco Casagrande
- **Chapter 6, Biosafety Risk Assessment:** Ryan Ritterson, Mark Kazmierczack, Molly Isbell (Signature Science), Christopher Hulme-Lowe (Signature Science), Erin Lauer, Mikaela Finnegan, Haley Krem, Audrey Cerles, Gautham Venugopalan, Ryan Handoko, Jacqueline Chu, Danielle Fields and Rocco Casagrande
- **Chapter 7, Biosecurity Risk Assessment of Acts Targeting a Laboratory:** Kavita Berger, Robert Stephan, Phillipe Mauger, Gautham Venugopalan and Rocco Casagrande
- **Chapter 8, Biosecurity Risk of GoF Information:** Landy Sun, Mikaela Finnegan, Phillipe Mauger, Craig Hooper and Rocco Casagrande
- **Chapter 9, Benefit Assessment:** Emily Billings, R. Alex Coots, Ryan Handoko, Phillipe Mauger, Ryan Ritterson, Rocco Casagrande, Casey Basham and Corey Meyer
- **Chapter 10, Proliferation of GoF Research:** Luba Katz (Abt Associates) and Rocco Casagrande
- **Chapter 11, Risk of Loss of Trust in Science:** Julia Homstad and Rocco Casagrande

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Also, we thank Barbara Johnson for her guidance on biosafety procedures in containment laboratories and Gerald Epstein and Wendy Hall of the Department of Homeland Security for a critical review of Chapter 8.

All cover images are from the CDC's Public Health Image Library: Top left, Microbiologist working at BSL3-E with H5N1 (image #8675), right, electron micrograph of MERS-CoV (image #18115) and bottom left, illustration of generic influenza virion (image #11877).

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1 Executive Summary

The analysis described in this report provides information for the NSABB to make recommendations about a general conceptual approach to the evaluation of Gain of Function studies and for the US government to formulate policy regarding Gain of Function (GoF) research. In this document, the term GoF is used in the same manner as the Framework for Conducting Risk and Benefit Assessments of Gain of Function Research—the "Framework". By design, this study was broad in its scope, intentionally assessing all of the traits and pathogens mentioned in the Framework to determine where risk lies. The conclusions of the risk assessment identify the pathogens and the enhanced phenotypes that increase risk of a pandemic and those that do not increase this risk. Similarly, the benefit assessment determines which experiments (regardless of their risk) have important and unique benefits.

This project is divided along the three major tasks, each of which requires a distinct data collection and analysis approach: 1) a risk analysis (RA) of accidents and natural disasters, 2) a biosecurity RA, and 3) a benefit assessment. The RA of accidents and natural disasters (called the biosafety RA for simplicity) leveraged sophisticated quantitative modeling of the probability and consequences of various events that lead to an outbreak, the ongoing transmission of the outbreak in humans, and the termination of the outbreak by public health measures or natural forces. The biosecurity RA includes an analysis of data from the intelligence and law enforcement community on malicious actors and an assessment of the efficacy of security measures at preventing or mitigating a hostile act. The biosecurity RA is delivered in two parts because risks posed by malicious acts targeting laboratories that conduct GoF required a different analytical approach than the assessment of the risk generated by the misuse of published GoF research. The benefit assessment identifies the gaps in scientific knowledge, public health, and medicine that GoF experiments could address. Moreover, this assessment discusses scientific and non-scientific barriers to the realization of these benefits.

1.1 Biosafety Risk Assessment

The Biosafety Risk Assessment is an estimation of the increase in risk to human health of outbreaks caused by modified strains of the influenza viruses and the coronaviruses released in an accident or natural disaster. This RA uses the word "coronavirus" to mean the coronaviruses that cause SARS or MERS and not the coronaviruses that cause the common cold. In every case, the increase in risk compared to wild type strains was provided to determine if GoF experiments could create pathogens that are more likely to cause laboratory acquired infections, to create a local outbreak, or to cause a global outbreak of greater consequence than those strains that evolved via natural forces. Note that although this study identified several types of risky, theoretical GoF experiments, many of these experiments have not been described in the literature. For example, no examples of researchers endeavoring to determine if seasonal influenza viruses could be made more transmissible were found. Moreover, some GoF studies are performed in highly attenuated strains, so that even though the risk of an outbreak increases if these strains were modified, risk is increasing from a very low level toward the level posed by wild type strains.

The main conclusion of the Biosafety RA is that a strain of influenza virus that is as transmissible as newly emerged pandemic strains WHILE producing a disease with a case fatality rate of more than 0.5% would pose more of a risk of a global pandemic than any wild type strain heretofore identified. No experiments that are likely to be conducted under the rubric of GoF research will drive risk more than this combination of traits or significantly increase the risk of a laboratory acquired infection. All other combinations of traits would lead to pathogens that have a lesser total risk than the wild type 1957 H2N2 pandemic strain. Increasing the transmissibility of the coronaviruses, while significantly increasing the

risk of work with those pathogens, still creates a pathogen that poses less of a risk of a global pandemic than the wild type 1957 influenza strain.

Another major finding of this risk assessment is that only a small minority of loss of containment events, which are rare in themselves, lead to a global pandemic. Only 0.5% of laboratory associated infections of seasonal influenza would seed a global pandemic, even assuming the accident was with a strain that has not circulated recently. If the strain released is currently in circulation, the spread of the outbreak is likely to be driven by travelers, not by laboratory accidents. If the released strain circulated recently, residual immunity is likely to curtail its spread. Only 1% of laboratory associated infections with wild type pandemic influenza strains would seed a global pandemic. Wild type strains of avian influenza and the diseases caused by the coronaviruses are insufficiently transmissible to have a significant chance of causing a global pandemic.[1]

Because seasonal influenza viruses are associated with a low case fatality rate, GoF experiments that increase this rate could significantly increase the global death toll from an outbreak, increasing risk. Developing seasonal influenza strains that are more transmissible than wild type strains (approximately as transmissible as pandemic strains) or that overcome residual immunity increases the probability that an outbreak would escape local control and exacerbates the consequences should a global outbreak be initiated (in terms of both morbidity and mortality). The creation of an antiviral resistant strain could increase the consequences of a global outbreak, but only in more economically developed countries where caches of these antivirals could be administered to a significant fraction of the infected population. A strain of seasonal influenza that can overcome protective vaccination could also increase the consequences of an outbreak in high income countries, which has the resources to vaccinate their population quickly. However, this phenotype is of concern only if immune evasion is afforded by means other than changing its antigenic properties, which is not a subject of current research in influenza. An unresolved question (which likely depends on the biology of the virus released and its similarity to currently circulating strains) is if the laboratory-associated outbreak of seasonal influenza would replace the annual toll of seasonal influenza by supplanting circulating strains or if it would add to this toll.

If GoF strains of seasonal influenza were manipulated at the BSL-3 instead of the BSL-2 level, risk overall may not increase much compared to work on wild type strains at BSL-2. That is, the rate of laboratory acquired infections is likely to decrease by three-fold, whereas any GoF phenotype (except for large increases in pathogenicity) increases risk by slightly more than three-fold.

In contrast to the several GoF manipulations that could increase the risk posed by seasonal influenza strains, only two lines of GoF research could create a strain of pandemic influenza that poses more risk of a global outbreak than a wild type strain (in this case, the 1957 H2N2 pandemic strain). The first is the manipulation of a strain of 1918 H1N1 pandemic influenza that is modified to evade residual immunity (or otherwise increase transmissibility to the same as a strain with novel antigenic properties). The second is the enhancement of pathogenicity (to that of 1918 H1N1 influenza) of a highly transmissible pandemic strain (such as 1957 H2N2 influenza). Imbuing 1957 H1N1 influenza with antiviral resistance can modestly increase the consequences of an outbreak, but only in countries with significant caches of antivirals. Enhancing viral growth in culture beyond that which is achievable in wild type strains (1E9 or 1E10/ml) increases the probability that a laboratory acquired infection would occur (by five- or 15-fold, respectively). However, it is doubtful if this phenotype is desirable or scientifically achievable because growth to 1E8 is sufficient for almost all purposes except the production of vaccines (using attenuated strains).

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[1] Althought the SARS outbreak spread to several locations on multiple continents, it was extinguished in all locations (each of which could be thought of as a new, local outbreak) and did not lead to millions of cases worldwide.

Wild type avian influenza is insufficiently transmissible amongst people to cause a global outbreak driven by the spread of the disease among humans. For this reason, no loss of containment event would lead to a global outbreak from a wild type strain. Because wild type strains of avian influenza cannot spread globally between people, the creation of strains that are human-transmissible would greatly increase the risk that such an outbreak could occur, which could cause millions of illnesses. The creation of a strain that is as transmissible as seasonal influenza would have a significant chance of sparking a global outbreak if a local outbreak were initiated. Assuming that the case fatality rates of the most pathogenic strains of avian influenza are inflated by the underreporting of mild illness in people, increasing the pathogenicity in humans could increase the consequences modestly. Adapting avian strains to humans without increasing transmissibility (thereby lowering the median infectious dose in people) actually decreases risk because while this manipulation increases in the probability that a single laboratory worker would become infected, it decreases the risk that birds would become infected through an accidental release via the solid waste stream, which could lead to thousands of human infections from contact with infected birds. No other GoF increases the risk posed by avian influenza.

Similarly, most estimates of the transmissibility of the coronaviruses consider these pathogens to be insufficiently transmissible and sufficiently susceptible to control measures such that a global pandemic has a very minimal chance of occurring. For this reason, increasing the transmissibility of the coronaviruses could significantly increase the chance of a global pandemic due to a laboratory accident. Because SARS-CoV is more transmissible than MERS-CoV, a relatively modest increase in transmissibility of SARS-CoV could increase risk, whereas MERS-CoV must be made significantly more transmissible to drive risk. That being said, even if these strains were modified to be as transmissible as pandemic influenza, the susceptibility to control measures of the outbreaks they cause would still contain a majority of the outbreaks initiated. Some researchers have posited that the transmissibility of wild type SARS-CoV is quite high. If they are correct, then increasing the transmissibility of SARS-CoV would not influence risk significantly because the risk of a global pandemic arising from an outbreak is already significant. Increasing the pathogenicity of these strains could also increase risk somewhat through the increase in global deaths expected, especially since most deaths from wild type strains are suffered by those with significant co-morbidities. However, if a coronavirus were modified such that it caused a global pandemic, their long incubation time and disease course[2] lead to a pandemic that unfolds over many years. The fact that the outbreak evolves slowly gives public health authorities more time to adapt and expand their efforts to further contain the outbreak than the modeling conducted in this assessment suggests. If a strain with enhanced growth properties was developed and samples with 1E9pfu/ml or 1E10pfu/ml were routinely manipulated in a laboratory, the risk of a laboratory acquired infection in a coronavirus laboratory would increase by up to ten-fold, respectively. However, it is uncertain if this phenotype is desirable or even achievable given that the wild type coronaviruses grow sufficiently well in culture.

The laboratory features and practices that most influence risk include the strict adherence to incident reporting and isolation protocols for laboratory workers. Minimizing the chance that a worker would violate either of these protocols can decrease the risk that an infected laboratory worker would create an outbreak by up to seven-fold when working with seasonal influenza virus or by ten-fold with the coronaviruses. Additionally, when working with the coronaviruses (which are more stable in the environment than the influenza viruses), protocols to minimize the hazard posed by the contamination of the hands (proper use of double-gloving and thorough hand-washing) can reduce the probability of an infection by nearly fifty-fold. The probability that workers themselves commit errors that generate the laboratory accident is more than one-hundred-fold greater than the probability that a mechanical failure leads to an accident. While this conclusion is self-evident, it underscores how extensive worker training

[2] As described in Chapter 4, although the incubation times of influenza virus and the coronaviruses overlap, the variance of the incubation time and disease course is much greater for the coronaviruses than for influenza.

prior to entry into a BSL-3 laboratory, the assignment of highly trained workers for critical safety tasks (such as the operation of autoclaves) and the identification and re-training of careless workers could all significantly improve safety.

The state of knowledge of the rates and consequences of human errors in life science laboratories is too poor to develop robust predictions of the absolute frequency with which laboratory accidents will lead to laboratory acquired infections. Using historical incidents (and lack thereof) as a guide, a rate (at the 90% confidence level) of a laboratory acquired infection every three to 8.5 years can be set across the 100 or so laboratories that study influenza and the coronaviruses in the US. Given that this study predicts that 0.4% of these infections would lead to a global pandemic (since most of these laboratories study seasonal influenza, and not pandemic influenza), work with wild type influenza viruses would lead a global pandemic once every 750-50,000 years. A significant risk of an outbreak would be caused only if the strain released in the accident were a seasonal influenza strain that has not recently circulated, however, this outbreak could lead to up to 4,000,000 deaths worldwide. It is uncertain if these deaths would supplement or supplant the yearly death toll from seasonal influenza. Conservatively, an infection with a pandemic influenza strain could be expected to lead to a global pandemic once every 560-13,000 years, causing up to 80,000,000 deaths if the strain used were as pathogenic as the 1918 pandemic strain (and as transmissible as the 1957 pandemic strain). Given that viruses were characterized much less than 100 years ago, it cannot be stated with certainty that these pathogens will be studied under similar containment conditions far enough into the future for an accident to be likely to occur even once. Avian influenza strains and coronavirus strains are insufficiently transmissible to cause a global pandemic.

If sufficient funding were available, GoF research could be conducted by up to approximately 40 research groups in the US because these groups have been performing, or have the capacity to perform, certain types of GOF experiments involving influenza, MERS, and SARS viruses. This maximum number is supported by the case studies examined which showed that a new discovery in virology may proliferate to as few as one and as many as 70 new groups around the world within 10-15 years.

1.2 Biosecurity Risk Assessment of Malicious Acts Targeting a GoF Laboratory

The purpose of this component of the biosecurity risk assessment is to provide NSABB with an assessment of the increased human health risk posed by a malicious act involving a GoF strain of the influenza- or coronaviruses compared to malicious acts involving wild type strains. The risk assessment involved five steps: 1) characterization of the threat, which includes an evaluation of historical incidents and malicious actor motivation and capability (the "offense"); 2) review of the current security policies and practices landscape that governs research with influenza, SARS-CoV, and MERS-CoV in the United States (the "defense"); 3) identification of plausible threats based on analysis of the "offense" and "defense"; 4) assessment of the potential for the plausible threats to cause infections in the local community or broader; and 5) comparison of possible pandemic consequences of plausible threats involving GoF viruses and non-GoF viruses. All of the data collected were used to assess the plausible threats facing laboratories that perform GoF research. These plausible threats serve as the most probable events that could lead to a loss of containment from a biosecurity incident. Therefore, they were used to focus the quantitative analysis of local and widespread infections on those acts that are the most plausible in today's laboratory security environment.

Based on historical incidents and an assessment of the security governance in the United States, the most likely malicious acts to be carried out in or on a containment laboratory include theft of virus stocks, experimental samples, equipment, or research animals; deliberate contamination of personal protective equipment or laboratory equipment of co-workers; deliberate compromise of the personal protective equipment or laboratory equipment of co-workers; and mixing of infected with uninfected samples or

animals outside proper containment. In addition, incidents involving bombs or active shooters may cause loss of containment if carried out inside or near the entrance of high containment laboratories in which GoF research is conducted.

In today's regulatory and security environment, the most plausible malicious acts taking place at high containment, research laboratories involves malicious insiders who have authorized access to the laboratories and virus(s) contained therein. Insiders may work alone or in coordination with an outside group. Their motivations range from emotional disturbances to ideological radicalization by domestic and transnational terrorist organizations. The likelihood that outsiders could gain access to a laboratory without insider assistance is low. Therefore, outsiders present a threat to the periphery of the research complex or building only, but not a significant threat to the high containment laboratory itself.

Only a handful of GoF traits significantly increase biosecurity risk after a malicious event targets a laboratory. For seasonal and pandemic influenza, the ability to overcome protective vaccination and antiviral resistance modestly increases risk by increasing the potential consequences in high income countries. There is no significant effect on risk if the global population is considered as a whole. For seasonal and pandemic influenza, increasing the transmissibility and ability to evade residual immunity significantly increases risk because outbreaks are more likely to occur, to escape local control, and will create more consequential global outbreaks. For avian influenza, increasing transmissibility greatly increases risk because this modification is required to spark a global outbreak of a disease by human-to-human contact, potentially infecting millions. Without this change, the hazard is restricted to those exposed to contaminated materials and infected birds, limiting the outbreak to thousands of cases at most. Increasing pathogenicity can modestly increase risk. Similarly, the wild type coronaviruses have a very small chance of sparking a global outbreak so increasing transmissibility greatly increases risk. Increasing pathogenicity can modestly increase risk.

When comparing the biosafety and biosecurity risks, a successful event that covertly infects the public (theft from an influenza laboratory of an infected animal, contaminated piece of equipment or viral stock) must occur once every 80-5,500 years for biosecurity event to have the same total risk as biosafety events. Given the frequency with which these malicious acts have occurred in the past, this analysis suggests that biosecurity considerations be given as much weight as biosafety issues.

1.3 Biosecurity Risk Assessment of GoF Information

The biosecurity RA of GoF information is based on the open-source literature covering desirable characteristics of biological agents and the scientific literature on GoF studies and non-GoF studies with significant dual-utility. The potential biosecurity information risk that could be generated by GoF information was assessed compared to what could be achieved through dual-use studies that do not rely on GoF research. It was then determined if the unique dual-use information resulting from GoF studies had already been published.

Little information risk remains from GoF research on the influenza viruses. Although the development of a highly-contagious, highly virulent strain of influenza presents significant biosecurity information risk, the methods to produce these strains have already been published and so no information risk remains. Moreover, the specific changes in the genome that lead to these traits have also been characterized and published, so an actor could reproduce the dual-use strains using reverse genetics. Similarly, information on how to develop strains of influenza viruses that grow well in culture/eggs or evade medical countermeasures or diagnostics has some dual-utility, but the methods to create these strains also have already been published. A modest information risk would be realized if researchers published methods to produce strains of influenza viruses that can produce more prolonged or chronic illness. Although this

manipulation is a possible enhancement of pathogenicity that can fall under the definition of GoF research, there is little scientific rationale to undertake these experiments. Hence, the possibility that this information risk will be realized is low. Another modest information risk inheres in the publication of methods to produce strains of influenza virus that are able to overcome protective vaccination even if the vaccine matches the serotype of the pathogen. Similar work has been published for other pathogens, but these pathogens have larger and more plastic genomes than the influenza viruses, so it is not known if similar manipulations could be successfully carried out in the influenza viruses.

Significant information risk would be realized by the publication of methods to create a highly transmissible SARS- or MERS-coronavirus that maintains its pathogenicity. Notably, without an animal model of transmissibility for these pathogens, this information risk is unlikely to be realized in the near future. A modest information risk inheres in methods to manipulate the genomic targets of a diagnostic assay for coronavirus infections without compromising the other desirable traits of the pathogen.

State actors (and the sub-state groups they sponsor) are currently the only groups with the resources, expertise, motivation, and time to leverage this dual-use information. These states could protect their own populace from a global pandemic by secretly stockpiling vaccines that are protective against their modified strain. For this reason, states would be more likely to produce modified influenza viruses than coronaviruses (because no vaccines exist for this type of agent) and would probably be uninterested in developing strains able to overcome any vaccine (as this strain would vitiate their comparative advantage). Sub-national malicious actors may obtain the capability to replicate some of the less complex GoF studies, but have so far not demonstrated any capacity to work with viral agents and little capacity for waging biological warfare in general. Highly skilled individuals trained in biology would be capable of replicating GoF studies, but are currently constrained greatly by a lack of material resources and time that are available typically only to well-funded companies and research institutions.

Finally, no information risks unique to GoF research were identified. Similar techniques to those used in GoF experiments could be leveraged for other pathogens that are not captured by the moratorium (and are therefore outside the initial GoF framework assessed in this document) to create a highly transmissible strain of an already deadly virus (like the Hendra and Nipah viruses) or to create a deadly strain of an already highly transmissible pathogen that has been modified to overcome protective vaccination (polio-, mumps- or measles-virus). Perhaps most worryingly, reverse genetics techniques could be used to synthesize smallpox virus if an actor has significant molecular biology skill, and this strain could be modified to overcome protective vaccination. Non-GoF pathogens could be used to produce effective, novel incapacitating agents by the modification of a highly contagious virus (polio-, mumps- or measles-virus) to overcome protective vaccination.

1.4 Benefit Assessment of GoF Research

The benefit assessment describes the potential benefits of GoF research involving influenza viruses and coronaviruses, relative to two different types of alternative approaches: alternative experimental approaches that can provide the same or similar information, and alternative scientific or technical innovations that may similarly benefit public health through completely different mechanisms. Notably, this assessment is limited to the evaluation of GoF experiments that have been published in the scientific literature.

Within the field of CoV research, GoF approaches in the following phenotypic categories were identified: enhanced pathogen production, altered host range, enhanced virulence, and evasion of therapeutics in development. GoF approaches that alter host range and enhance virulence uniquely enable the development of animal model systems that recapitulate human disease pathogenesis, which are critical for

establishing the safety and efficacy of candidate vaccines and therapeutics and for the study of disease pathogenesis mechanisms. GoF approaches that enhance virulence are also uniquely capable of demonstrating that live attenuated vaccines (LAVs) do not recover virulence upon growth *in vivo*, an important aspect of safety testing of candidate LAVs. Of note, this particular type of experiment simply increases the human health risk of the attenuated strain to approach that of wild type strains. GoF approaches that enhance virulence represent the most efficient and effective strategy for discovering novel virulence factors, which may be good targets for new therapeutics. However, several alternative strategies for the development of new therapeutics are being actively pursued and have also shown promise. GoF approaches that lead to evasion of therapeutics in development are critical for the development and regulatory approval of new therapeutics. Because these therapeutics are not yet widely available, no increase in human health risk is posed by resistant strains. GoF approaches that alter host range and enhance virulence provide unique benefits to study cross-species adaptation and pathogenicity, but alternative approaches may also be used. Of note, this adaptation to a new host typically attenuates virulence in the original host (in the case of SARS and MERS-CoV, humans).

Within the field of influenza research, GoF approaches in the following phenotypic categories were identified: enhanced pathogen production, mammalian adaptation and enhanced transmissibility, enhanced virulence, evasion of vaccines or therapeutics, and evasion of existing natural or induced immunity. Across all GoF phenotypes, GoF approaches provide unique benefits to the study of the mechanistic basis of the phenotype under study as well as the evolutionary mechanisms driving acquisition of that trait, though alternative approaches may also be used. Alternative approaches have stringent limitations for the study of mechanisms underlying mammalian transmissibility of animal influenza viruses, as animal flu viruses that efficiently transmit in humans do not exist in nature. GoF approaches that enhance virus production are uniquely critical for the current ability to produce sufficient and timely influenza vaccines for seasonal flu epidemics and flu pandemics and represent the only strategy for improving existing vaccine production capabilities in the near-term. Of note, GoF approaches used in vaccine production attenuate an otherwise pathogenic strain while enhancing its growth properties. GoF approaches that enhance the infectivity, transmissibility, and virulence of animal flu viruses inform pandemic risk assessments of circulating influenza viruses, which guide downstream decision-making about investments in pre-pandemic vaccine development and other pandemic preparedness initiatives. Specifically, GoF approaches are uniquely critical for strengthening the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence, which can be used to infer phenotype from sequence for the risk assessment. In general, molecular marker data moderately contribute to the overall risk associated with a particular virus. However, molecular marker data play an important role in rapid risk assessments when novel flu viruses first emerge in human populations due to the early availability of viral sequence data. These risk assessments facilitate more rapid initiation of response activities such as pre-pandemic vaccine development. Of note, realization of this benefit is subject to significant advancements in the state of knowledge about mechanisms underlying mammalian adaptation, transmissibility, and virulence, as well as improvements to global public health laboratory infrastructure. In addition, molecular marker data guide selection of viruses used as the basis of pre-pandemic vaccines. GoF approaches that enhance the infectivity and virulence of influenza viruses are also used to develop animal models that support the study of disease pathogenesis and medical countermeasure (MCM) development. GoF approaches that lead to evasion of therapeutics in development are critical for the development and regulatory approval of new therapeutics. Of note, the acquisition of resistance to novel classes of therapeutics is not expected to confer cross-resistance to existing antivirals (i.e., adamantanes or neuraminidase inhibitors). Thus, when these experiments involve drug candidates within new classes of therapeutics, which are not yet widely available, no increase in human health risk is posed by resistant strains. However, similar approaches using licensed therapeutics inform therapeutic recommendations for seasonal influenza infections and pandemic preparedness initiatives for high-risk animal influenza viruses, but phenotypic approaches for antiviral sensitivity testing are also used for these purposes. GoF approaches that lead to evasion of vaccines are uniquely

capable of determining whether viruses can acquire mutations to escape neutralization of candidate broad-spectrum or universal influenza vaccines, a critical aspect of testing the potential field efficacy of vaccines in development. Most of these experiments involve next-generation influenza vaccine candidates targeting epitopes other than the globular head domain of the hemagglutinin (HA) protein, the target of current influenza vaccines. Given that the globular head domain of HA is the immunodominant protein of influenza viruses and that these next-generation vaccines are not yet widely available, strains that can overcome the protection afforded by these vaccines are expected to pose a minimal increase in human health risk relative to wild type strains. GoF approaches that lead to evasion of existing natural or induced immunity have potential to improve the efficacy of seasonal influenza vaccines, but this benefit is subject to advancements in the state of knowledge about the mechanistic basis of antigenic drift as well as expansion of sequencing capabilities across public health laboratories involved in global influenza surveillance. Finally, GoF studies involving reassortment, which may lead to one or more phenotypic changes, are uniquely capable of providing information that can be used to prioritize community-level interventions aiming to prevent opportunities for co-infections that could lead to the generation of reassortant viruses with phenotypic properties of concern.

2 Overview and Purpose

The overarching purpose of conducting the risk/benefit analysis (RBA) is to provide information for the NSABB to make recommendations about a general conceptual approach to the evaluation of Gain of Function studies, and for the US government to formulate policy regarding Gain of Function (GoF) research. In this document, the term GoF is used in the same manner as the Framework for Conducting Risk and Benefit Assessments of Gain of Function Research—the "Framework".[3] By design, this study was broad in its scope, intentionally assessing all of the traits and pathogens mentioned in the Framework to determine where risk lies. The conclusions of the risk assessment should point to the pathogens and the enhanced phenotypes that would increase risk of a pandemic and those that do not increase this risk. Similarly, the benefit assessment determines which experiments (regardless of their risk) have important and unique benefits. That being said, this study was not so broad as to assess the risk posed by experiments that could create pandemic pathogens that do not all within the Framework. Specifically, as discussed in Chapter 8, other pathogens that lie outside the framework could be manipulated to cause a global outbreak. Also, other traits of the influenza viruses and coronaviruses could be manipulated that would alter their pandemic potential (such as their environmental stability, which could significantly increase their risk of causing a laboratory acquired infection, and the probability that patients could transmit the disease to others prior to the onset of symptoms).

The specific goals of this assessment is to provide evidence on how particular GoF experiments affect the following possibilities:

- That an outbreak caused by a laboratory accident may occur,
- That an outbreak caused by a laboratory accident may increase in severity or extent,
- That a hostile actor may misuse the materials or information generated,
- That future or ongoing disease outbreaks or attacks could be prevented or mitigated, and
- That the life-science research in general would be advanced.

2.1 Organization of the Project

This project is divided along the three major tasks, each of which requires a distinct data collection and analysis approach: 1) a risk analysis (RA) of accidents and natural disasters, 2) a biosecurity RA and 3) a benefit assessment. The RA of accidents and natural disasters (called the biosafety RA for simplicity) leveraged sophisticated quantitative modeling of the probability and consequences of various events that lead to an outbreak, the ongoing transmission of the outbreak in humans and the termination of the outbreak by public health measures or natural forces. The biosecurity RA requires an analysis of data from the intelligence and law enforcement community on malicious actors and an assessment of the efficacy of security measures at preventing or mitigating a hostile act. The biosecurity RA is delivered in two parts because risks posed by malicious acts targeting laboratories that conduct GoF required a different analytical approach than the assessment of the risk generated by the misuse of published GoF research. The benefit assessment requires an understanding of the gaps in scientific knowledge, public health and medicine that GoF experiments could address. Moreover, this assessment requires the identification of scientific and non-scientific barriers to the realization of these benefits.

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[3] Framework for Conducting Risk and Benefit Assessment of Gain-of-Function Research: Recommendations of the National Advisory Board for Biosecurity. May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

2.2 Time Horizon

The life sciences are advancing extremely rapidly such that techniques commonly use today were unheard of a few years ago, and the findings are being applied to more and more facets of life. Hence, the state of science decades from now is impossible to predict; to ground the work in real science, the RBA is constrained by a five year time horizon. This time horizon is necessary because the approach is data driven and the future state of research protocols is unknowable, especially how these changes will affect stocks of pathogen, containment measures, public health measures, and gaps in scientific knowledge, public health and medicine. New modes of scientific inquiry could obviate GoF research or open up new opportunities for its application. New laboratory techniques could greatly reduce the chance that an accident would occur or that any infections may happen. Of relevance to biosecurity, the malicious actors who may misuse the fruits of GoF research in the far future may have motives or capabilities much different from those of today's actors.

Specifically, all risks are considered in a five year time horizon. In contrast, the follow-on benefits of a scientific discovery that is produced in a five year time-frame will be considered even if these benefits are realized further into the future. This expanded time-horizon for benefits is necessary because basic science finds its application in the field years after its discovery and some regulatory processes require more than five years by themselves before products borne of a scientific discovery can be used.

2.3 Interpreting the Results of the RBA

In this study, GoF phenotypes are analyzed individually so that the NSABB can understand how any particular anticipated change would affect risk in isolation. In reality, many of the phenotypes considered by the framework are inextricably linked. For example, a component of transmissibility of seasonal influenza in human populations is the protection afforded by exposure to similar strains in the past. For this reason, the ability to overcome residual immunity influences transmissibility. Similarly, adaptation to a host is a necessary component of being transmissible in that host. A strain that is adapted to a host is likely to grow to a higher titer in cells derived from that host and produce a higher titer infection in a living host. High titer infections may often lead to a greater amount of viral shedding, and so these phenotypes are likely related to transmissibility.

The modeling completed enables a complete assessment of how any combination of parameter values that describe the pathogen and control measures influences risk, however, all possible combinations of these values and their influence on risk cannot be shown concisely in a report. Instead, static slices through this very complex risk space are taken and shown as two-dimensional figures in this report that explore the effect of changing one parameter while allowing all others to vary.

This study examines the risk should a GoF experiment lead to a pathogen with particular traits. In a quantitative framework, these traits must be described numerically (such as a specific increase in the reproductive number of the outbreak or the median infectious dose). However, quantitatively translating empirical studies of transmission in animals to epidemiological predictions for human populations is impossible. That is, increases in transmissibility in ferrets in isolators cannot be linked to a specific increase in the reproductive number for outbreaks in human populations. Therefore, it is unknown if the enhanced transmissibility observed in GoF experiments done to date would significantly change the risk of an outbreak. Only one component of the transmissibility of a virus in a human population is the biology of the virus and the host because humans may change their behavior to reduce the risk of contact during a particularly worrying outbreak. In fact, a recent study estimates that the ferret model of influenza

can be used to explain only 66% of the variation in transmissibility in humans observed across subtypes.[4] Instead, this RBA can simply determine how particular increases in transmissibility of a pathogen causing a human outbreak would influence risk. The feasibility of achieving any particular phenotype via GoF research is a question of science.

Lastly, this study uses the actuarial definition of risk (risk is the product of probability and consequences of a bad event). Wherever possible, this study clearly describes how aspects of GoF research influence risk by increasing the probability that an outbreak would occur and/or by increasing the consequences should it occur. In this way, readers can use this document to inform their calculations based on other possible definitions of risk (the probability that a bad event of any consequence occurs, for example).

[4] Bühnerkempe, MG et al. "Mapping influenza transmission in the ferret model to transmission in humans" *eLife*, 2015, e07969.

3 Overall Methodology

This project is divided along three major tasks, each of which requires a distinct data collection and analysis approach: 1) a risk analysis (RA) of accidents and natural disasters, 2) a biosecurity RA, and 3) a benefit assessment. The RA of accidents and natural disasters (called the biosafety RA for simplicity) requires sophisticated quantitative modeling of the probability and consequences of various events that lead to an outbreak, the ongoing transmission of the outbreak in humans and the termination of the outbreak by public health measures or natural forces. The biosecurity RA, which considers acts that originate in a GoF laboratory and the misuse of the information generated by GoF research, requires an analysis of historical data on malicious actors and an assessment of the efficacy of security measures at preventing or mitigating a hostile act. The benefit assessment requires an understanding of the gaps in scientific knowledge, public health and medicine that GoF experiments could address. Moreover, this assessment requires the identification of scientific and non-scientific barriers to the realization of these benefits.

3.1 RA of Accidents and Natural Disasters

If this assessment is to inform a system for the evaluation of future research, the RA of accidents and natural disasters must provide risk information about research that has yet to be initiated in locations that have yet to be identified. This RA must also consider work with wild type pathogens that does not fall under the umbrella of GoF research. Unfortunately, the experiments to manipulate pathogens with pandemic potential (PPP), the resultant phenotypes, the biosafety features of the laboratory, and the environment around the laboratory all could significantly influence risk. To cover the entire landscape of experiments, phenotypes, containment measures, and environments, we took a parametric approach to risk modeling. That is, we determined how changing any attribute of the pathogen, experiment, laboratory, or environment would affect risk and then bound this assessment in science by assigning real examples to particular values. For example, we assessed how the transmissibility of an influenza virus affects risk of an outbreak from arbitrarily small values of transmissibility through arbitrarily large values. In this manner, we provide information on how transmissible an influenza virus must be in order for risk to increase significantly. We then compared this "break point" to the transmissibility of known influenza viruses to provide context on the feasibility of novel strains reaching this level of transmissibility. A similar approach was taken with all GoF phenotypes. Similarly, biocontainment aspects and features of the environment were explored for their influence on risk and we highlight those features or qualities of the environment that may significantly influence risk.

Using this approach, we considered biosafety risk by its component parts: the *probability* that an event would occur that would lead to an infection outside the laboratory, the *probability* that the infection would lead to an outbreak that seeds a global epidemic, and the *consequences* of the global epidemic.

The RA of accidents and natural disasters began with the accidents and natural disasters themselves. Of all the events that COULD lead to a loss of containment that could befall a laboratory, we chose to quantitatively model those that were either identified as high-risk in previous laboratory risk assessments, cited as frequent causes of accidents in laboratories in incident reports or those that are uniquely relevant to GoF studies. These events included high-probability, low-consequence events (like spills), low-probability, high-consequence events (like earthquakes), and "maximum reasonably foreseeable events". Events that are both low-probability and low-consequence were considered but not modeled further because they will, by definition, not contribute to risk.

Because of the routine use of Fault Tree Analysis (FTA) in a Probabilistic Risk Assessment (PRA) framework in the estimation of risks arising from accidents and natural disasters creating technological hazards (accidents and disasters striking nuclear power plants and the chemical supply chain, for example),[5] we applied a similar methodology here. In FTA, the probability that a specific hazard is generated via a series of connected failures is estimated. This analysis method is most commonly used to understand how systems can fail and to identify the best ways to reduce risk. To explore the uncertainty in parameter values and the variety of possible paths through a fault tree, we employed Monte Carlo simulations, in which repeated, random draws of possible paths and parameter values are sampled to obtain an aggregate realization of risk. For each incident (and for all incidents in aggregate) we obtained a probability that various types and sizes of releases occur. The PRA estimated how frequently each release occurs and how much pathogen (or how many infected animals or people) is released. Releases could occur via aerosol, via an infected worker or animal, or via a contaminated worker.

For each release type, a different modeling approach was used. For releases that create an aerosol, we used an atmospheric dispersion model to determine how many people or animals are exposed to what dose of pathogen. Dose-response models were used to determine how many people or animals become infected. For releases of contamination on the body or clothing of a laboratory worker, a stochastic, Markov chain model was developed to determine how many people (if any) or how many animals (if any) become contaminated after being touched by the initial contaminated worker. For events that caused the infection of a laboratory worker, we used a stochastic model to determine if the worker violates the various procedural and medical monitoring protocols to determine the probability of initiating an outbreak in the community. As discussed further below, animal escapes were found to drive risk by escaping containment features within the laboratory and infecting workers who can then leave the laboratory instead of the animal escaping the laboratory entirely.[6]

Once a person was infected outside of the laboratory, we modeled the nascent local outbreak using a branching process model, which captures the fact that small outbreaks can be extinguished by stochastic factors and also public health measures, some of which may be unique to the communities around the laboratories. Branching process models are stochastic models that calculate how many individuals every contagious person infects in each generation. In our model, the probability of infecting a certain number of new individuals is determined by the transmissibility of the pathogen (described by the parameter R) and the variation in transmissibility between individuals (described by the parameter k) and modified by public health control measures. We used this model to determine the probability that any given outbreak would extinguish or grow beyond local control, given the properties of the pathogen, starting conditions (how many of what type of people are infected), and control measures.

Once an outbreak escapes local control, it seeds outbreaks throughout the world. We modeled the illnesses and deaths that occur in each region of the world using the HHS-BARDA Interactive Influenza Model, an SEIR model that considers the effect of the young and the elderly in the ongoing spread of the disease given contact rates between workplace, school, and home populations. Although this study did not attempt to evaluate the efficacy of public health response measures in detail, these measures must be captured in our RA because some GoF phenotypes may vitiate some control measures more than others (for example, the ability to overcome protective vaccination) and lead to a change in relative risk.

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[5] For example, see Vesely et al. Fault Tree Handbook (NUREG-0492), U.S. Nuclear Regulatory Commission, January 1981, http://pbadupws.nrc.gov/docs/ML1007/ML100780465.pdf and Center for Chemical Process Safety, Guidelines for Hazard Evaluation Procedures, April 2008.

[6] The biosecurity section (Chapter 7) discusses an event that involves malicious actors stealing infected animals from a laboratory. In this event, the malicious actor is assumed to be infected by carrying the infected animals, and the infection of this person drives subsequent outbreak risk.

For pathogens that are transmissible amongst birds only, if a bird is infected outside of the laboratory, we assumed that the outbreak escapes local control and that human health consequences are suffered based on the consequences of past avian influenza outbreaks. This simple approach was taken because not enough data is available to support more robust modeling of the interplay of wild birds, domestic birds and humans for outbreaks caused by entirely novel, avian-transmissible pathogens that cannot be transmitted amongst people. If an avian-origin strain is modified to be transmissible in mammals, it was modeled as any other human-transmissible pathogen, as described above.

If a global epidemic is not triggered, consequences were tallied from the number of people infected in the laboratory or in the smaller-scale outbreak in the locality. Comparing the risk posed by GoF research to the risk posed by unmodified pathogens provides an understanding of which specific GoF experiments may lead to a significant increase in biosafety risk.

Because the risk of a laboratory accident is proportional to the number of laboratory workers manipulating dangerous strains of pathogens, we also characterized the proliferation potential of GoF research in the US should the funding pause be lifted. We assessed the potential interest and capability to perform GoF research in the US by an analysis of the scientific literature. We also examined funding availability and the sufficiency of containment space to perform the work. Lastly, we identified three cases of scientific discoveries in virology and traced their proliferation over the following years to provide additional insight.

3.2 Biosecurity RA of Malicious Acts Targeting a Laboratory

In the risk assessment of malicious acts targeting a laboratory, also known as the semi-quantitative biosecurity assessment, we compared the motivations and capabilities of a variety of malicious actors to the defensive systems arrayed against them to prevent the malicious act. Should a malicious act lead to the loss of containment, its consequences were modeled in the same manner as in the biosafety RA above.

No unclassified information describing the threats to research laboratories that store or study GoF influenza, SARS, or MERS-CoV virus is available. Therefore, to identify the types of acts that may target a GoF laboratory, our approach involved examining historical incidents involving life science laboratories and hospitals, evaluating the motivations and capabilities of malicious actors, and determining if and how existing security measures affect the likelihood of success of a malicious act. Plausible threats facing laboratories that study or store GoF virus(s) were extrapolated from this assessment. From this assessment, we can compare quantitatively how a malicious act would have different consequences if a GoF laboratory was targeted instead of a laboratory studying only wild type pathogens.

To organize our biosecurity data collection effort, we developed a matrix of malicious actors, acts, and consequences. This matrix was reviewed by officials from the law enforcement and intelligence communities to ensure that we captured all plausible combinations that could threaten biosecurity. We then populated this matrix with data drawn from historical events that involved malicious acts in laboratories in the US or overseas. This historical analysis provides an evidence-based method to understand, in a qualitative way, the probability that an event would occur and the type of resources these malicious actors bring to bear when targeting a laboratory.

To assess the capabilities of preventing malicious acts, we investigated the literature on legal authorities and systems supporting biosecurity and analyzed these authorities and systems for gaps that could be exploited by malicious actors. We also interviewed biosecurity stakeholders at institutions performing relevant research to understand specific systems in place at these locations.

We then compared the data on the motives and capabilities of the malicious actors to the capabilities of systems preventing their access to develop a series of qualitative scenarios that represent the "highest risk" biosecurity events. The consequences of these events were modeled using the methodology laid out in the biosafety RA as described above.

3.3 Biosecurity RA of GoF Information

In this assessment, we identified those GoF studies that, if published, would provide useful information over what is already published in the scientific literature to a malicious actor seeking to create a biological weapon. To perform this assessment, we first determined what is possible for a malicious actor to achieve using unmodified agents so that we can identify how GoF pathogens could afford *additional* capabilities to an adversary. We then characterized the state of the science regarding the enhancement of all traits described in the NSABB GoF risk and benefit framework to understand to what degree methods already exist in the literature that speak to the creation of modified strains of influenza viruses and coronaviruses with phenotypes attractive to malicious actors. In this way, we identified GoF research that would provide uniquely valuable information to a malicious actor for misuse over the body of dual-use research that already exists. Also, we identified if dual-use information already in the literature requires a particularly challenging technical approach in order to ascertain if an information risk could be suffered via the publication of an easier experimental route to the same product. Lastly, we used open-source information to understand if this unpublished dual-use information is actually desired by various malicious actors and characterized the technical skill, sophistication, and resources required for those actors to leverage this information.

3.4 Benefit Assessment

The approach to the benefit assessment is founded on the concept that the benefits of scientific research derive from applications of new scientific information or products to gaps in knowledge, public health, medicine, and other societal issues. To that end, a multi-step process was used to identify the potential benefits of GoF research.

1. A foundation for the analysis was established by independently:
    a. characterizing the expected scientific information and products derived from GoF studies of potential concern involving influenza viruses and coronaviruses (Pathogens with Pandemic Potential, or "PPPs"), and

    b. identifying gaps in scientific knowledge about PPPs and gaps in public health and medical capabilities related to the prevention and control of PPP outbreaks.

2. The scientific information/products derived from GoF research were mapped ("crosswalked") to the gaps in scientific knowledge, public health, and medicine,

3. Alternate experimental approaches and/or other scientific or technical innovations ("alt-GoF" approaches) that could address the same gaps were identified,

4. The barriers to the realization of GoF and alt-GoF benefits were evaluated,

5. The unique benefits of GoF research were identified by comparatively analyzing the benefits afforded by GoF research versus alternative approaches, in light of the barriers to the realization of each approach,

6. The potential for the unique benefits of GoF research to be globalized was assessed, and

7. Benefits to the production of influenza vaccines were quantitatively evaluated.

This analysis of GoF benefits was guided by the benefits of GoF research and associated benefit critiques proposed by infectious disease researchers and other GoF stakeholders during public meetings about GoF research and through perspectives published in scientific journals. Each proposed benefit and benefit critique was examined in detail through interviews with stakeholders involved in conducting scientific research, including PPP researchers and non-PPP researchers, and stakeholders involved in translating research insights into public health practice and policy. Additionally, this list of proposed benefits and benefit critiques was expanded upon through further analysis of the scientific literature. Each proposed benefit was then validated – the "crosswalk" of proposed benefits to gaps – through examination and analysis of the scientific literature (for benefits to scientific knowledge) or through interviews with stakeholders in public health and MCM development who are directly involved in applying the data or agents generated through GoF research to public health practice and policy and MCM development/production. The validation analysis included an assessment of the relevance and validity of all benefit critiques previously identified. Importantly, this analysis leveraged the evaluation of public health systems to understand the process by which the immediate applications of GoF research ultimately reduce human morbidity and mortality caused by influenza viruses and coronaviruses. Taken together, this analysis resulted in the identification of GoF research outputs with validated applications to scientific knowledge, public health, and medicine as well as an understanding of their downstream benefits to the health of human populations.

Some alternative experimental approaches or other scientific/technical innovations (hereafter referred to as "alt-GoF" approaches) may pose less risk than GoF studies but yield the same or similar benefits. As GoF studies comprise a subset of all research activities involving PPPs, this analysis focused exclusively on those alt-GoF approaches capable of targeting the same gaps in scientific knowledge and public health as GoF approaches. The potential benefits of alt-GoF studies were identified through the same process as for GoF studies: a crosswalk of the research outputs of alt-GoF studies to gaps in scientific knowledge, public health, and medicine related to PPPs. Importantly, in addition to alternative experimental approaches, alt-GoF approaches also include those scientific and technical innovations that address the same public health gaps that GoF can address but through a completely different mechanism. To complement the analysis of the net risks associated with the conduct of GoF research relative to research involving wild type pathogens, the benefit assessment highlights those types of GoF studies that may provide *unique* benefits to scientific knowledge, public health, and medicine relative to alternative approaches.

One of the most challenging aspects of weighing the risks and benefits of GoF research is that there is a temporal mismatch between the risks and the benefits of the research – the risks are assumed at the time the research is conducted, while the benefits to public health and medicine *may* accrue in the future. To enable the comparison of risks and benefits, the benefit assessment provides data on the likelihood that the potential benefits of GoF research will be realized by describing the barriers to the realization of the benefits. Two types of barriers were explored: scientific barriers and non-scientific barriers. Scientific barriers arise from uncertainties in the state of the science and/or in the meaning of the scientific outcomes of GoF studies, which may influence the nature and limit the scope of the benefit. Scientific barriers were identified through analysis of the scientific literature and interviews with infectious disease researchers. Non-scientific barriers include other technical innovations and regulatory factors that are essential for translation of the research, as well as gaps or inefficiencies in downstream aspects of the public health process that may limit the ultimate impact of the research application on human health. To identify non-scientific barriers, the gap analysis of public health and medical capabilities related to the prevention and control of PPP outbreaks was leveraged. Finally, the type of resources needed to

overcome or circumvent each barrier was defined, including advancements in scientific knowledge, improvements to public health infrastructure, and other factors, which serves as a proxy for the likelihood and timing of the realization of the benefits.

Whether risks and benefits are equally distributed across populations is also an important consideration in any risk-benefit comparison. To inform NSABB's deliberations on this issue, the benefit assessment qualitatively assessed the globalization potential of the identified GoF benefits, through analysis of historical case studies examining the globalization of similar benefits and through review of relevant USG policies on resource and information sharing. Benefits related to the production of influenza vaccines are amenable to quantitative analysis. This analysis leveraged models developed for the biosafety RA above to parametrically explore how changes in the availability of influenza vaccines can mitigate morbidity or mortality during seasonal flu epidemics and flu pandemics.

4 Background Information on Influenza Viruses, the Coronaviruses and GoF Research

4.1 Influenza Viruses

Throughout this report, the terms pandemic, seasonal, and avian are used. Seasonal influenza viruses include the strains of the H1N1 and H3N2 subtypes that cause morbidity on an annual basis. Pandemic influenza viruses include the 1918 H1N1, 1957 H2N2, 1968 H3N2 and 2009 H1N1 strains, which spread rapidly at least partially because the population had very little immunity. The 2009 H1N1 strain continues to circulate seasonally since its emergence, and is properly classified as both a pandemic strain and a seasonal strain (also the morbidity and mortality caused by this strain is more similar to seasonal strains than any of the previous pandemic strains). Similarly, today's seasonal H1N1 strains are descendants of the 1918 H1N1 strain and the seasonal H3N2 strains are descendants of the 1968 strain, so the distinction between pandemic and seasonal strains is one of timing (today's novel pandemic strain is tomorrow's seasonal strain).

Avian influenza strains are strains of influenza that are transmissible only amongst animals other than humans, especially birds. If an avian strain is modified to become transmissible in humans, we still call this an avian strain because of the characteristics of its wild type parents. Generally, this report is concerned with the highly pathogenic strains of the H5 and H7 subtypes.

4.1.1 Biology of Influenza

4.1.1.1 Overview

Influenza is a single-stranded, negative-sense RNA virus of the *Orthomyxoviridae* family. There are three types of influenza viruses—A, B, and C—that have a common genetic ancestry, but distinct genetic characteristics. Influenza type A can infect a variety of animal hosts and is further divided into subgroups based on its surface proteins. Type B viruses have a more limited host range with limited variation; influenza C causes only mild symptoms in humans and does not contribute to outbreaks.[7]

4.1.1.2 Virus Structure

The influenza genome is divided into eight RNA segments that encode viral proteins essential to the functionality of the virus. Each is folded into a rod-shaped, double-helical ribonucleoprotein complex (RNP).

[7] Centers for Disease Control and Prevention. Types of Influenza Viruses. http://www.cdc.gov/flu/about/viruses/types.htm. Last Update 2014. Accessed May 2015.

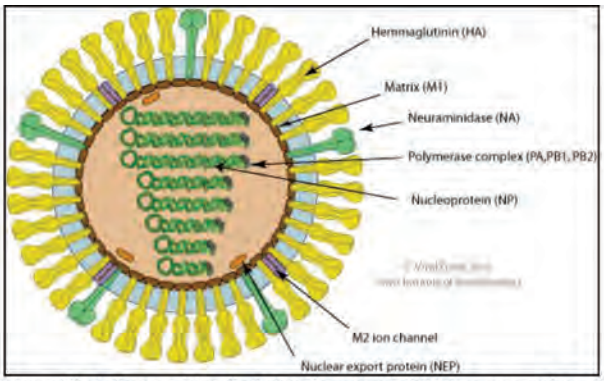

Figure 4.1. A cartoon of the influenza virion displaying the segmented RNA genome and encoded proteins, as reproduced from Le Mercier.[8]

The RNP contains viral RNA encapsulated by nucleoprotein (NP) and bound to a trimeric RNA polymerase, comprised of one polymerase acidic (PA) and two polymerase basic (PB1, PB2) subunits. The RNP is responsible for directing RNA replication, transcription, and transport as well as genome reassortment and packaging. Nuclear export proteins (NEP) also facilitate intracellular transport.

Matrix proteins, M1, surround the RNPs and NEPs. The lipid bilayer envelope encloses the virion with hemagglutinin (HA), neuraminidase (NA), and matrix ion channel (M2) proteins embedded into its membrane. HA glycoproteins facilitate virus binding and entry whereas NA proteins promote virus budding. HA recognizes the sialic acid moieties on host cells and ensure proper binding in preparation for endocytosis. NA proteins possess sialidase activity to release newly replicated virus from and prevent virus aggregation on the host cell. M2 ion channel proteins are vital for pH regulation during viral replication.[9,10,11]

4.1.1.3 Antigenic Variation

All influenza viruses are classified by type and strain. Influenza A viruses are also classified by their HA and NA subtype—e.g., H1N1, H3N2, H5N1. Influenza is a relatively simple RNA virus, yet is able to continuously elude host immune systems through antigenic drift. Influenza's RNA polymerase is prone to replication errors, resulting in frequent point mutations in antibody binding sites on HA and NA proteins. These amino acid changes have the potential to affect the conformation of surface proteins, and hence, the binding of host antibodies. Although these mutations are minor and random, accumulation over time can lead to a new strain of virus that is no longer neutralized by the host immune system, even after vaccination or prior infection.

Antigenic drift occurs in both influenza A and B. Influenza A viruses, however, can also evolve through a much more abrupt process referred to as antigenic shift, which is the result of genomic reassortment.

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8 Le Mercier P (2010) Influenza virus A. SIB Swiss Institute of Bioinformatics, ViralZone. Retrieved from http://viralzone.expasy.org/all_by_species/6.html.
9 Bouvier NM, Palese P (2008b) The biology of influenza viruses. *Vaccine* 26, Supplement 4: D49-D53
10 Shaw ML *et al* (2008) Cellular proteins in influenza virus particles. *PLoS Pathog* 4: e1000085
11 Tsai KN, Chen GW (2011) Influenza genome diversity and evolution. *Microbes Infect* 13: 479-488

The segmented feature of the virus genome enables the entire HA or NA segment to be replaced with a new segment from a different influenza virus. By altering the surface proteins, the infectivity of the virus is also altered and a new phenotypic subtype is formed. The HA protein is more likely to be reassorted, but both HA and NA subtypes have variable antigenicity.

Genomic reassortment occurs as a result of co-circulation of different subtypes of influenza A and co-infection of a host. When a host is infected with two influenza strains, viral replication in the nucleus may cause mixing of genetic material. Influenza is prone to genetic mixing due to its multiple-stranded genome (that is, a single newly budded virus particle could package RNA strands from two different parental strains). This mixing can result in a significant change of the antigenic properties of the virus, termed antigenic shift, and could generate a virus to which hosts have no existing immunity and, therefore, is the source of pandemic outbreaks. After a virus undergoes antigenic shift, it continues to experience antigenic drift. Both antigenic drift and antigenic shift are responsible for influenza's evolution and survival.[12,13]

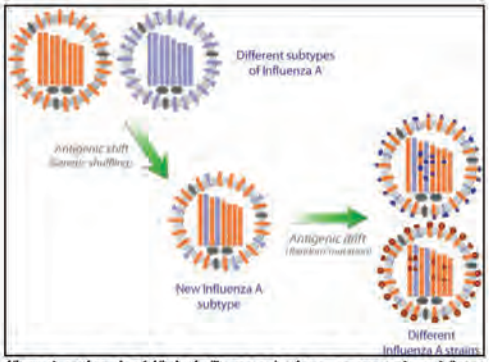

Figure 4.2. Antigenic drift and antigenic shift in influenza A virus, as reproduced from the WHO Collaborating Centre for Reference and Research on Influenza.[14]

4.1.1.4 Host Range

Influenza types, subtypes, and strains have a distinct and sometimes overlapping set of host organisms that they can effectively infect, called their host range or host tropism. For influenza viruses, sialic acid receptor specificity, temperature, and pH at the site of infection are the main determinants of host range.

Receptor specificity largely determines the host tropism of a virus. HA proteins bind to host glycosylated receptors with sialic acid moieties; different HA subtypes have preferences for different bond structures. HA in avian viruses binds only to the α-2,3 isoform whereas in human-adapted viruses, the α-2,6 linkage is preferred (Figure 4.3). Either HA type, however, will bind in swine because the species possesses cells with both sialic acid moieties. For this reason, swine are considered "mixing vessels" that provide an

12 Carrat F, Flahault A (2007) Influenza vaccine: the challenge of antigenic drift. *Vaccine* 25: 6852-6862
13 Bouvier NM, Palese P (2008a) The biology of influenza viruses. Ibid. 26 Suppl 4: D49-53
14 WHO Collaborating Centre for Reference and Research on Influenza. About Influenza. http://www.influenzacentre.org/aboutinfluenza.htm. Last Update Accessed October 2015.

opportunity for reassortment. Reassortment and evolution in intermediate hosts allow for emergence of new virus types. Figure 4.4 below shows the species adapted to different HA and NA subtypes.[15,16]

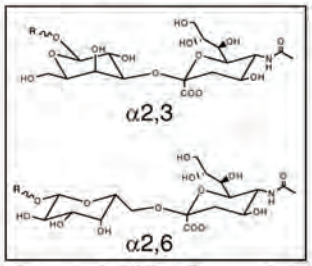

Figure 4.3. Chemical structures of α-2,3- and α-2,6-linked glycans, with the terminal sialic acid and galactose, for binding to influenza viruses, as reproduced from Xu et al.[17]

15 Xu R *et al* (2010) Structure, receptor binding, and antigenicity of influenza virus hemagglutinins from the 1957 H2N2 pandemic. *J Virol* 84: 1715-1721

16 Medina RA, Garcia-Sastre A (2011) Influenza A viruses: new research developments. *Nature reviews Microbiology* 9: 590-603

17 Xu R *et al* (2010) Structure, receptor binding, and antigenicity of influenza virus hemagglutinins from the 1957 H2N2 pandemic. *J Virol* 84: 1715-1721

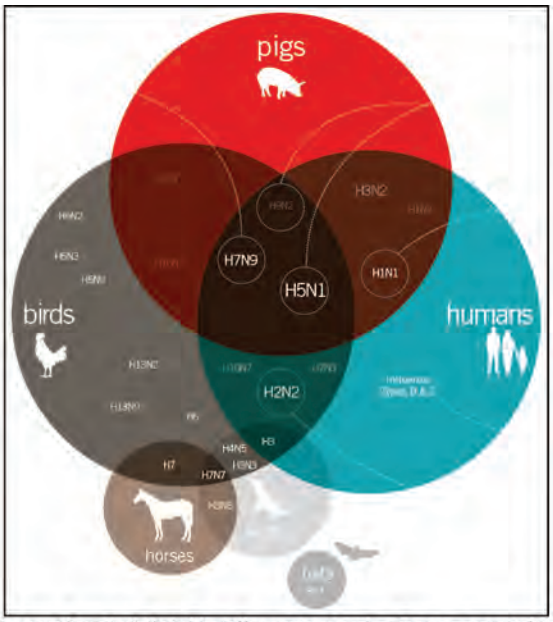

Figure 4.4. Venn diagram of species infected by influenza types and subtypes as reported by the CDC and WHO, adapted from McCandless et al. Size of text represents human fatality rates and lighter text shows the virus rarely infects humans.[18]

For effective transmission to another host, the virus must be able to efficiently replicate in the temperature of the new host and the site of infection in each host has a distinct temperature range. In birds, infection occurs in the gastrointestinal tract at around 40 degrees Celsius. In swine, influenza targets the respiratory tract at approximately 39 degrees Celsius. The upper respiratory tract in humans is typically around 33 degrees Celsius whereas the lower respiratory tract reaches 37 degrees Celsius. The lower respiratory tract also has α-2,3 moieties, which can be bound by avian strains. The elevated temperature, in combination with avian compatible viral receptors, presents the opportunity for a non-human-adapted virus to infect a human. Although rare, this is a source of unexpected species crossover, creating a variant influenza strain.[19,20]

HA glycoproteins facilitate viral infection of a host cell through pH-induced membrane fusion and some level of host tropism is determined by the pH of the infection site in various hosts. Change in pH may render the virus ineffective at transferring its genome into the host cell by causing the virus to release its genome at less proximity to the nucleus or once lysosomes have matured to degrade the genome. Either

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18 McCandless D, Hollowood E. Influ-Venn-Za. Who can catch which flu? http://www.informationisbeautiful.net/visualizations/which-flu-virus/. Last Update April 2013. Accessed October 2015.

19 Medina RA, Garcia-Sastre A (2011) Influenza A viruses: new research developments. *Nature reviews Microbiology* 9: 590-603

20 Causey D, Edwards SV (2008) Ecology of avian influenza virus in birds. *J Infect Dis* 197 Suppl 1: S29-33

will inhibit viral infection and replication. Viral adaption to a new host species requires a pH shift for effective membrane fusion.[21]

Currently only H1, H2, and H3 subtypes can easily cause human-to-human transmission. The emergence of a new subtype capable of human to human transmission is expected to cause a major pandemic because the population will have no pre-existing immunity.

4.1.2 Influenza Epidemiology[22]

Influenza is an acute viral infection characterized by the rapid onset of disease and brief symptomatic period. The virus can cause mild to severe respiratory illness in human hosts; some infections cause minor respiratory symptoms while others result in hospitalization and occasionally, death. Unresolved cases are usually associated with other chronic conditions and can develop into additional complications such as pneumonia and bronchitis.

4.1.2.1 Incubation Period

The incubation period is the time between when an individual is exposed to a pathogen and when the first symptom manifests. During the incubation period, most infected individuals cannot transmit the infection to others; therefore, longer incubation periods equate to a slower outbreak development. Incubation periods vary for seasonal, pandemic, and severe pandemic influenza.

4.1.2.1.1 Seasonal Influenza

Several papers were identified that describe the incubation periods observed in seasonal influenza infections. The literature suggests an incubation period duration ranging from one day to seven days (Supplemental Information Table 1). The most common incubation period found within the literature was two days with a mean incubation period of 63 hours or 2.6 days.[23,24,25,26,27,28,29]

4.1.2.1.2 Pandemic Influenza

Incubation periods for pandemic influenza are reported to be slightly longer than those seen in seasonal influenza. Since there is little to no data on the incubation period of other pandemic strains, data from the 2009 H1N1 outbreak were evaluated. Four sources from the 2009 H1N1 pandemic reported data on the length of incubation periods.

The H1N1 data suggest a range of incubation periods similar to the range seen in seasonal influenza.

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21 Mair CM *et al* (2014) Receptor binding and pH stability — How influenza A virus hemagglutinin affects host-specific virus infection. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1838: 1153-1168

22 Much of the data and sources discussed below were drawn from a previous study completed for the Defense Threat Reduction Agency by Gryphon Scientific called *Influenza Modeling Parameters*.

23 Alford RH *et al* (1966) Human influenza resulting from aerosol inhalation. *Experimental Biology and Medicine* 122: 800-804

24 Burnet F, Foley M (1940) The Results of Intranasal Inoculation of Modified and Unmodified Influenza Virus Strains in Human Volunteers. *Medical Journal of Australia* 2: 655-659

25 Couch RB *et al* (1971) Correlated studies of a recombinant influenza-virus vaccine. III. Protection against experimental influenza in man. *Journal of Infectious Diseases* 124: 473-480

26 Macdonald P, Lyth JC (1918) INCUBATION PERIOD OF INFLUENZA. *Br Med J* 2: 488

27 Moser MR *et al* (1979) An outbreak of influenza aboard a commercial airliner. *American journal of epidemiology* 110: 1-6

28 Armstrong C, Hopkins R (1921) An epidemiological study of the 1920 epidemic of influenza in an isolated rural community. *Public Health Reports (1896-1970)*: 1671-1702

29 Lessler J *et al* (2009) Incubation periods of acute respiratory viral infections: a systematic review. *The Lancet infectious diseases* 9: 291-300

However, these data suggest a mean incubation period of 4.2 days and median of 4.1 days instead of the two day incubation period most commonly seen in seasonal influenza.[30,31,32,33,34]

4.1.2.2 Infectious Period

The infectious period is the disease stage when an infected individual can transmit their disease to others. Currently, however, there is no definitive way to determine when an individual infected with influenza virus is contagious. The most widely accepted method of determining contagiousness is by measuring viral shedding. Under this method, an individual is deemed infectious when they begin shedding virus and stops being infectious when the viral shedding ends. The infectious period of seasonal and pandemic influenza is seemingly the same.

4.1.2.2.1 Seasonal and Pandemic Influenza

Data on when individuals infected with influenza stop shedding virus are extremely limited. The few availably papers typically assume that viral shedding begins at the onset of symptoms, and report only the average time after symptom onset when viral shedding ceases at 5.9 days.[35,36] Only Doyle et al. reported the distribution of viral shedding durations in addition to average duration.[37] In this study individuals were experimentally infected with influenza H1N1 virus (a pandemic strain) and monitored daily for viral shedding. All infected individuals shed virus for a minimum of three days after onset of symptoms, and a small percentage of individuals shed for eight or more days (see Supplemental Information on influenza disease course). However, more than 50% of those infected shed virus for six or seven days. The study also provided evidence that viral shedding occurred before symptoms were displayed, which would increase the total time of shedding. No other sources were available on the duration of viral shedding for influenza or to confirm viral shedding before symptoms.

4.1.3 Asymptomatic Infections

A small portion of individuals infected with influenza virus never get clinically ill. These asymptomatic individuals are infected with influenza, shed virus, and therefore have the potential to transmit to others, but never develop symptoms.

4.1.3.1 Seasonal Influenza

Several studies examined the percent of asymptomatic seasonal influenza infections. Data from three papers, Lau et al., Loeb et al., and Suess et al., were included in our analysis (Supplemental Information on influenza disease course). These three studies all used the same method for defining an asymptomatic

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30 Cao B *et al* (2009) Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. *New England Journal of Medicine* 361: 2507-2517

31 Li H, Wang SX (2010) Clinical features of 2009 pandemic influenza A (H1N1) virus infection in chronic hemodialysis patients. *Blood Purif* 30: 172-177

32 Tuite AR *et al* (2010) Estimated epidemiologic parameters and morbidity associated with pandemic H1N1 influenza. *Canadian Medical Association Journal* 182: 131-136

33 Wang C *et al* (2012) Epidemiological and clinical characteristics of the outbreak of 2009 pandemic influenza A (H1N1) at a middle school in Luoyang, China. *Public Health* 126: 289-294

34 Ghani A *et al* (2009) The Early Transmission Dynamics of H1N1pdm Influenza in the United Kingdom. *PLoS currents* 1: RRN1130

35 Carrat F *et al* (2008) Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *American journal of epidemiology* 167: 775-785

36 Lau LL *et al* (2010) Viral shedding and clinical illness in naturally acquired influenza virus infections. *Journal of Infectious Diseases* 201: 1509-1516

37 Doyle WJ *et al* (1998) Effect of rimantadine treatment on clinical manifestations and otologic complications in adults experimentally infected with influenza A (H1N1) virus. *J Infect Dis* 177: 1260-1265

infection.[38] Individuals were considered to be asymptomatic if they were actively shedding influenza virus but were not experiencing any upper respiratory infection symptoms. Individuals that were exposed to influenza through a close contact (usually a family member) were monitored for influenza viral shedding to determine if an infection had occurred. Infected individuals were then monitored to determine whether or not symptoms developed. The three studies suggest that 13% of individuals infected with seasonal influenza virus experience an asymptomatic infection. [39,40,41]

4.1.3.2 Pandemic Influenza

Only one paper was identified that examined asymptomatic pandemic influenza infections. During the 2009 H1N1 pandemic, Papenburg et al. used the same techniques described by Lau et al., Loeb et al., and Suess et al. in which asymptomatic individuals that shared a household with symptomatic individuals were monitored for viral shedding.[42] Papenburg et al. found 9.4% of individuals that shed H1N1 influenza virus remained symptom free.

4.1.4 Symptomatic Infections

An influenza diagnosis encompasses a variety of symptoms that can manifest in different combinations within each individual. Many symptoms are shared by seasonal and pandemic influenza, but some are only produced by more severe pandemic infections. Symptoms associated with seasonal influenza include chills, cough, diarrhea, fatigue, fever, headaches, myalgia, nasal congestion, rhinorrhea, sore throat, and vomiting. Additionally, pandemic influenza can also cause abdominal pain, bronchospasms, chest pain, confusion, conjunctivitis, loss of appetite, nosebleeds, and seizures. Not every individual will experience all of the symptoms for each disease.

4.1.4.1 Seasonal and Pandemic Influenza

The prevalence of some influenza symptoms are also age dependent.[43] For example, children with seasonal influenza are significantly more likely to experience vomiting than are those who are 60 years and older. The prevalence of each influenza symptom varies between children, adults, and the elderly during seasonal and pandemic outbreaks (Supplemental Information on influenza disease course).

Data on the prevalence of symptoms were obtained from observational influenza studies and from the control subjects of anti-influenza neuraminidase inhibitor clinical trials. These studies recorded the number and/or percentage of people experiencing an influenza infection and the specific symptoms they developed. No data on pandemic influenza in the elderly was identified. [44]

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38 A 2008 paper by Carrat et al. also reviewed this topic; however, it did not explain how "asymptomatic" infections were defined and was therefore excluded from our analysis. Carrat F *et al* (2008) Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *American journal of epidemiology* 167: 775-785

39 Lau LL *et al* (2010) Viral shedding and clinical illness in naturally acquired influenza virus infections. *Journal of Infectious Diseases* 201: 1509-1516

40 Loeb M *et al* (2012) Longitudinal study of influenza molecular viral shedding in Hutterite communities. *Journal of Infectious Diseases* 206: 1078-1084

41 Suess T *et al* (2012) Comparison of shedding characteristics of seasonal influenza virus (sub) types and influenza A (H1N1) pdm09; Germany, 2007–2011. *PloS one* 7: e51653

42 Papenburg J *et al* (2010) Household transmission of the 2009 pandemic A/H1N1 influenza virus: elevated laboratory-confirmed secondary attack rates and evidence of asymptomatic infections. *Clinical Infectious Diseases* 51: 1033-1041

43 Centers for Disease Control and Prevention. Flu Symptoms & Severity. Retrieved from http://www.cdc.gov/flu/about/disease/symptoms.htm. Last Update September 2014. Accessed May 2014.

44 Cox NJ, Subbarao K (1999) Influenza. *The Lancet* 354: 1277-1282

4.1.5 Mortality

4.1.5.1 Seasonal Influenza

Almost all infected patients will fully recover. A small portion of illnesses, however, will end in death. Thompson et al. in 2003 analyzed and abstracted seasonal influenza data compiled by the National Center for Health Statistics (NCHS) from 1990-1998. These data were then used to estimate the rate of influenza-associated deaths by age groups (Supplemental Information on influenza disease course). The percent excess mortality of infected individuals ranges from 0.0002% for those between five and 49 years old to 0.02% for those older than 64.[45]

4.1.5.2 Pandemic Influenza

The mortality rate of pandemic influenza is both difficult to estimate or predict due to the limited number of past pandemic outbreaks. It is estimated that approximately 500 million people were infected with the 1918 Spanish flu and 50 to 100 million people perished as a result of infection.[46] During the 2009 H1N1 pandemic, there were anywhere from 43 to 89 million cases of influenza with resultant 9,000 to 18,000 deaths.[47] The percentage of influenza infections that resulted in mortality was approximately 5% during the 1918 pandemic and less than 0.05% during the 2009 pandemic (Supplemental Information on influenza disease course).

4.2 The SARS- and MERS-coronaviruses

Throughout this report, our use of the term "coronaviruses" or "CoVs" refers specifically to SARS-CoV, MERS-CoV, and SARS/MERS-like bat CoVs such as HKU4 and HKU5. Note, the four human coronaviruses that cause mild to moderate respiratory illnesses such as the common cold or croup (coronaviruses HKU1, OC43, 229E, and NL63) were not evaluated because these are not considered in the NSABB GoF Framework.

4.2.1 Biology of the Coronaviruses

Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) are caused by SARS-associated (SARS-CoV) and MERS-associated coronavirus (MERS-CoV), respectively. Coronaviruses are positive sense, single-stranded RNA viruses. They are the largest of all RNA viruses, comprised of approximately 30 thousand nucleotides. Due to the length of its genome, coronaviruses can be less dependent on cellular proteins than other RNA viruses, enabling easier cross-species transmission.

Three groups of coronaviruses have been identified, all with distinct genetic and serological identities. While they are both beta-coronaviruses, MERS-CoV is from lineage B while SARS-CoV belongs to

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45 Thompson WW *et al* (2003) Mortality associated with influenza and respiratory syncytial virus in the United States. *Jama* 289: 179-186

46 Taubenberger JK, Morens DM (2006) 1918 Influenza: the mother of all pandemics. *Emerging infectious diseases* 12: 15-22.

47 National Institute of Allergy and Infectious Diseases. (2011) Pandemic Flu History. Department of Health & Human Services, Washington, DC.

lineage C.[48] Aside from SARS-CoV and MERS-CoV, coronaviruses that can infect humans cause common colds, lower respiratory tract infections, and diarrhea.[49,50]

4.2.2 Genome Structure of the Coronaviruses

The long SARS-CoV genome is broken into five major open reading frames (ORF), which are sections of nucleotides responsible for coding a peptide (Figure 4.1). Beginning at the five prime end, the first two ORFs, 1a and 1b, comprise two-thirds of the genome and encode the viral replicase genes, which encode proteins that are responsible for viral genome replication in the host cell. Further down the genome, ORFs encode genes for the structural proteins of SARS-CoV: spike (S), envelope (E), membrane (M), and nucleocapsid (N). These characterized ORFs are interspaced between several other ORFs that encode accessory genes. While the exact role of accessory genes is unknown, they are believed to contribute to viral pathogenesis and not replication.[51,52]

MERS-CoV has a similar genome structure, including viral replicase genes and structural proteins, as SARS-CoV.[53]

4.2.2.1 Structural Proteins and Particle Structure

The M glycoprotein is responsible for virus assembly. M proteins are the most abundant transmembrane protein in the viral envelope, where they interact with N proteins. N proteins self-associate to helically encapsidate the viral RNA and form the ribonucleoprotein complex (RNP). Together with M proteins, these N proteins mediate incorporation of the genome into budding virions for release. N proteins are highly immunogenic and their interaction with host cell proteins establishes pathogenicity. Envelope (E) proteins are a hydrophobic integral membrane proteins that serve as viroporins, which form ion channels in the envelope and therefore, play a central role in virus morphogenesis and assembly. E proteins are also credited with preserving the membrane's curvature for particle stability and infectivity.

Lastly, spike proteins are transmembrane fusion proteins responsible for effective viral entry into host cells. The N-terminal domain (S1) facilitates target receptor binding while the C-terminal domain (S2) ensures proper viral fusion. The S1 domain differs between coronavirus types and is largely responsible for host range. [54] Activated spike proteins induce the host immune response, including antibody neutralization and are the major antigenic determinants of MERS-CoV and SARS-CoV. [55,56,57,58]

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[48] Hilgenfeld R, Peiris M (2013) From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. *Antiviral Res* 100: 286-295

[49] Satija N, Lal SK (2007) The molecular biology of SARS coronavirus. *Ann NY Acad Sci* 1102: 26-38

[50] Li W *et al* (2006) Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. *J Virol* 80: 4211-4219

[51] Kopecky-Bromberg SA *et al* (2007) Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. Ibid. 81: 548-557

[52] Satija N, Lal SK (2007) The molecular biology of SARS coronavirus. *Ann NY Acad Sci* 1102: 26-38

[53] Coleman CM, Frieman MB (2013) Emergence of the Middle East respiratory syndrome coronavirus. *PLoS Pathog* 9: e1003595

[54] Li F (2015) Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *J Virol* 89: 1954-1964

[55] Siu YL *et al* (2008) The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. Ibid. 82: 11318-11330

[56] Tan YJ *et al* (2005) Characterization of viral proteins encoded by the SARS-coronavirus genome. *Antiviral Res* 65: 69-78

[57] Satija N, Lal SK (2007) The molecular biology of SARS coronavirus. *Ann NY Acad Sci* 1102: 26-38

[58] Li W *et al* (2006) Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. *J Virol* 80: 4211-4219

The virion contains one copy of the viral genome encapsidated by N proteins in an RNP (Figure 4.5). A viral envelope surrounds the virion with structural proteins S, E, and M embedded in its membrane. Coronavirus has a crown-like appearance due to the protruding club-shaped spike proteins.[59]

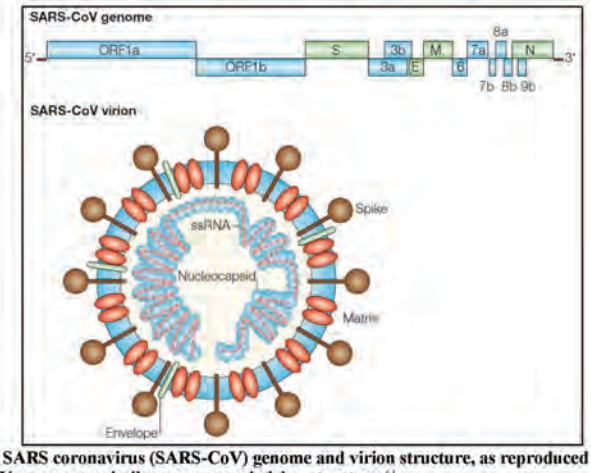

Figure 4.5. The SARS coronavirus (SARS-CoV) genome and virion structure, as reproduced from Perlman et al. [60] MERS-CoV possesses a similar genome and virion structure.[61]

4.2.3 Diversity of the Coronaviruses

Coronaviruses evolve rapidly, similar to all RNA viruses because polymerase infidelity results in amino acid mutations that alter transcription and potentially translation. Although these genetic mutations are minor and random, accumulation over time can lead to a new strain of virus. Some believe that the unusually large coronavirus genome leads to more mutations. Other studies have shown, however, that the genome encodes additional RNA processing and editing enzymes to correct for polymerase errors.[62,63,64]

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59 Ibid.

60 Perlman S, Dandekar AA (2005) Immunopathogenesis of coronavirus infections: implications for SARS. *Nature reviews Immunology* 5: 917-927

61 Coleman CM, Frieman MB (2013) Emergence of the Middle East respiratory syndrome coronavirus. *PLoS Pathog* 9: e1003595

62 Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology* 84: 3134-3146

63 Li W *et al* (2006) Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. Ibid. 80: 4211-4219

64 Dudas G, Rambaut A (2015) MERS-CoV recombination: implications about the reservoir and potential for adaptation. *bioRxiv*

The coronavirus genome is also prone to homologous recombination. Recombination allows for genetic exchange between different virus strains during coinfection. The natural process facilitates cross-species transmission and the generation of new coronavirus species.[65,66,67]

Recombination can affect all viral proteins, but variation in the spike protein has a considerable effect on the virus due to its role in viral entry and host range. Changes in virulence, species transmission patterns, and host range are often a result of spike protein recombination.[68,69]

4.2.4 Host Range of the Coronaviruses

4.2.4.1 SARS-CoV

Before infecting humans, SARS-like CoVs infected an array of other animal species, including bats, palm civets, monkeys, domestic cats, raccoons, and ferrets. Bats are the virus's natural reservoir, however, palm civets are credited as the amplifying host that transmitted SARS-CoV to humans.[70]

Host specificity of SARS-CoV is heavily influenced by receptor recognition and hence, its spike protein. Viral sequencing suggests that the spike protein experienced heavy positive selection at the onset of the SARS outbreak.[71] Clinical data supports this premise, as SARS-CoV became increasingly pathogenic and transmissible among humans as the epidemic progressed; virus evolution through mutations to the spike protein were the likely cause.[72,73]

SARS-CoV entry is mediated by angiotensin I converting enzyme 2 (ACE2), the host cell receptor (Figure 4.2). Ordinarily ACE2 regulates host blood pressure. Host susceptibility to the SARS coronavirus is dependent on the binding affinity between the virus and the host-specific ACE2. Only two residue-altering mutations in the ACE2 gene were necessary to overcome the species barrier between palm civets and humans leading to sustained human infection.[74,75]

ACE2 is primarily found on ciliated cells in the lung epithelia, which explains the tropism of SARS-CoV to the lungs and the resultant respiratory illness. These receptors have also been detected in the heart, colon, and kidneys.[76,77] The absence of this receptor in muscle, blood or skin cells suggest that there is very little risk of infection if SARS-CoV is introduced in a cut.

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65 Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology* 84: 3134-3146

66 Li W *et al* (2006) Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. Ibid. 80: 4211-4219

67 Dudas G, Rambaut A (2015) MERS-CoV recombination: implications about the reservoir and potential for adaptation. *bioRxiv*

68 Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology* 84: 3134-3146

69 Li W *et al* (2006) Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. Ibid. 80: 4211-4219

70 Li F (2013) Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Res* 100: 246-254

71 Sheahan T *et al* (2008) Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. *Journal of virology* 82: 2274-2285

72 ibid.

73 Li F (2013) Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Res* 100: 246-254

74 Sheahan T *et al* (2008) Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. *Journal of virology* 82: 2274-2285

75 Li F (2013) Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Res* 100: 246-254

76 Sheahan T *et al* (2008) Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. *Journal of virology* 82: 2274-2285

77 Li F (2013) Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral Res* 100: 246-254

4.2.4.2 MERS-CoV

The MERS-CoV spike protein, just as in SARS-CoV, largely determines the host range of the virus. Dipeptidyl peptidase 4 (DPP4), also known as CD26, was recently identified as the host receptor for viral entry. DPP4 is a widely expressed cellular protease tasked with assisting immune responses, glucose metabolism, and apoptosis. The glycoprotein is found on many cellular surfaces, including the kidneys, lungs, small intestines, and liver, which accounts for the virus's ability to cause systemic infection and shock.[78,79]

MERS-CoV infects a larger range of animals than does SARS-CoV. Transmission has occurred through close contact between humans and animals, most likely dromedary camels or bats.[80] The MERS coronavirus can also infect primates, horses, and goats, but is ineffective in smaller mammals such as ferrets, hamsters, and mice.[81] Host susceptibility to MERS is dependent on the binding affinity between the virus and the host-specific DPP4. Differences have been detected in DPP4 glycoproteins among mammals that may alter such affinity.[82]

There are no similarities between the structure or sequence of DPP4 and ACE2, the host receptor for SARS-CoV, which explains the distinct host ranges among the two coronaviruses. Further research suggests that differences in expression levels and locations of the receptors may account for the viruses' difference pathogenesis.[83]

4.2.5 SARS-CoV Epidemiology

SARS is an acute viral respiratory illness that develops into severe pneumonia. It is a contagious and virulent disease. Without treatment, the pneumonia may lead to respiratory failure and death.[84]

4.2.5.1 Incubation Period

The incubation period is the time between when an individual is exposed to a pathogen and when the first symptom manifests. During the incubation period of SARS and MERS, infected individuals probably cannot transmit the infection to others; therefore, longer incubation periods equate to a slower outbreak development.

The incubation period of SARS is was found to vary significantly between patients and during the 2003 pandemic, between countries. According to the World Health Organization (WHO), most countries experienced a median incubation period of four to five days and mean of four to 6 days with a minimum of one day and maximum of 14 days reported.[85] The primary literature is rich with studies on the incubation periods of SARS cases (Supplemental Information on CoV disease course). The literature

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78 Abdel-Moneim AS (2014) Middle East respiratory syndrome coronavirus (MERS-CoV): evidence and speculations. *Arch Virol* 159: 1575-1584

79 Peck KM *et al* (2014) Coronavirus Host Range Expansion and Middle East Respiratory Syndrome Coronavirus Emergence: Biochemical Mechanisms and Evolutionary Perspectives. *Annual Review of Virology*

80 Penttinen PM *et al* (2013) Taking stock of the first 133 MERS coronavirus cases globally--Is the epidemic changing? *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 18

81 Peck KM *et al* (2014) Coronavirus Host Range Expansion and Middle East Respiratory Syndrome Coronavirus Emergence: Biochemical Mechanisms and Evolutionary Perspectives. *Annual Review of Virology*

82 Wang N *et al* (2013) Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Res* 23: 986-993

83 Ibid.

84 The Centers for Disease Control and Prevention (2004) Basic Information about SARS *Fact Sheet*

85 The World Health Organization, Severe Acute Respiratory Syndrome (SARS) Epidemiology Working Group (2003a) Consensus document on the epidemiology of severe acute respiratory syndrome (SARS).

suggests a mean incubation period of 5.18 days and median of four days.[86,87,88,89,90] Donnelly found that 95% of patients experience onset of symptoms within 14.22 days.[91] The literature findings generally support the published reports from the WHO but presented a range of one to 18 days, capturing the variability of the SARS incubation period. No definitive explanations exist for the cause of the distribution of incubation period is so widespread, but difficulty identifying exposure, varying infectious doses, and multiple exposures are possible causes. Route of transmission may also affect the incubation period, but it is unclear why or how.[92,93,94,95,96]

4.2.5.2 Infectious Period

The infectious period is the disease stage when an infected individual can transmit the disease to others. The most widely accepted method of determining contagiousness is measuring viral shedding. Under this method, an individual is deemed infectious when they begin shedding virus and stops being infectious when the viral shedding ends.

Data on viral shedding and the infectious period of SARS is very limited. Cori et al. modeled the average infectious period in SARS patients to be 9.3 days.[97] Available literature agrees that viral shedding is low within the first few days following infection, meaning contagiousness is also low. The available research from Isakbaeva et al., Cheng et al., and Peiris et al. suggests the viral shedding peaks between day ten and day 14 following infection (Supplemental Information on CoV disease course).[98,99,100] However, Isakbaeva et al. also found viral shedding to persist for 26 days in a patient in the United States.[101] The Centers for Disease Control and Surveillance (CDC) recommends that while SARS patients are most contagious during their second week of illness, they should also limit contact with others for ten days after symptoms subside.[102]

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86 Donnelly CA *et al* (2003) Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* 361: 1761-1766
87 Meltzer MI (2004) Multiple contact dates and SARS incubation periods. *Emerg Infect Dis* 10: 207-209
88 Varia M *et al* (2003) Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada. *CMAJ* 169: 285-292
89 Hsu LY *et al* (2003) Severe acute respiratory syndrome (SARS) in Singapore: clinical features of index patient and initial contacts. *Emerg Infect Dis* 9: 713-717
90 Leung GM *et al* (2004) The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: an analysis of all 1755 patients. *Ann Intern Med* 141: 662-673
91 Donnelly CA *et al* (2003) Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* 361: 1761-1766
92 Ibid.
93 Meltzer MI (2004) Multiple contact dates and SARS incubation periods. *Emerg Infect Dis* 10: 207-209
94 Varia M *et al* (2003) Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada. *CMAJ* 169: 285-292
95 Hsu LY *et al* (2003) Severe acute respiratory syndrome (SARS) in Singapore: clinical features of index patient and initial contacts. *Emerg Infect Dis* 9: 713-717
96 Leung GM *et al* (2004) The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: an analysis of all 1755 patients. *Ann Intern Med* 141: 662-673
97 Cori A *et al* (2009) Temporal variability and social heterogeneity in disease transmission: the case of SARS in Hong Kong. *PLoS computational biology* 5: e1000471
98 Isakbaeva ET *et al* (2004) SARS-associated coronavirus transmission, United States. *Emerg Infect Dis* 10: 225-231
99 Cheng PK *et al* (2004) Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. *Lancet* 363: 1699-1700
100 Peiris JS *et al* (2003) Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Ibid. 361: 1767-1772
101 Isakbaeva ET *et al* (2004) SARS-associated coronavirus transmission, United States. *Emerg Infect Dis* 10: 225-231
102 The Centers for Disease Control and Prevention (2004) Basic Information about SARS *Fact Sheet*

4.2.5.3 Symptoms

SARS typically begins with influenza-like symptoms, including high fever, fatigue, sore throat, headache, and myalgia. Some patients also experience diarrhea, dry cough, and shortness of breath. As SARS progresses, most cases will develop into pneumonia.[103]

According to Donnelly et al., the most common symptom is fever, with 94% of cases reporting this symptom to the Hong Kong Department of Health. Influenza-like symptoms were second most common at approximately 72% of illnesses. Less than one quarter of patients displayed gastrointestinal symptoms such as diarrhea, vomiting, and abdominal pain. Approximately 88% of illnesses presented fever plus one other symptom.[104] Without treatment, the pneumonia may lead to respiratory failure and death.

4.2.5.4 Mortality

The overall case fatality-rate of the SARS outbreak is estimated at 11%, according to the WHO. Between age groups, rates vary from 0%-50%.[105] Christian et al. assessed the range to be between 0%- 40% with an overall fatality rate of 9.6%.[106] The high rate of mortality of SARS in the elderly is not accurately captured by the overall case-fatality rate. Although infection rates were similar, Wang et al. asserts that the fatality rate in those over 75 years old was 38% whereas no deaths occurred in those under 24 years.[107] Similarly, analysis by Donnelly et al. determined the case-fatality rate for persons under 60 years old to be 6.8% while the rate for over 60 years was 55%.[108] Advanced age is the most influential risk factor for SARS-associated death. In addition to age, diabetes mellitus and hepatitis B virus infection are other risk factors for death.

4.2.6 MERS-CoV Epidemiology

Middle East Respiratory Syndrome (MERS) is a respiratory infection that can develop into an acute severe respiratory illness. Many cases end in death, although most who succumb suffer from significant co-morbidities.

4.2.6.1 Incubation Period

According to the CDC, the incubation period of MERS can range from two to 14 days with a median of five days. As of July 2015, the WHO supports a median incubation period of 5.5-6.5 days.[109] Several additional literature sources were identified that describe the incubation period. Analysis by Cowling et al., Assiri et al., and Park et al. determined that the median incubation period of MERS is 6.07 days with a range from two to 15 days (Supplemental Information on CoV disease course). The literature, the WHO,

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103 Ibid.
104 Donnelly CA *et al* (2003) Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* 361: 1761-1766
105 World Health Organization. Alert, verification and public health management of SARS in the post-outbreak period. http://www.who.int/csr/sars/postoutbreak/en/. Last Update August 14, 2003. Accessed July 2015.
106 Christian MD *et al* (2004) Severe acute respiratory syndrome. *Clin Infect Dis* 38: 1420-1427
107 Wang MD, Jolly AM (2004) Changing virulence of the SARS virus: the epidemiological evidence. *Bull World Health Organ* 82: 547-548
108 Donnelly CA *et al* (2003) Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* 361: 1761-1766
109 The World Health Organization (2015b) Summary of Current Situation, Literature Update and Risk Assessment. *Middle East respiratory syndrome coronavirus (MERS-CoV)* 15: 1-7

and the CDC recommendations concur that most patients begin experiencing symptoms within the first week of contact with the MERS coronavirus.[110,111,112]

4.2.6.2 Infectious Period

Limited and inconclusive information is available on the infectious period of MERS. Patients are considered infectious while they are shedding the virus, but time-specific data is lacking. They are not contagious during the incubation period, however patients may continue to shed virus after symptoms have subsided.[113,114,115] A study by Memish et al. reported that 76% of studied cases were still shedding virus 12 days after symptoms appeared. Additionally, analysis showed that sicker patients and those with significant comorbidities shed MERS-CoV for a longer period of time than standard cases.[116]

4.2.6.3 Symptoms

MERS symptoms range from mild to severe; patients display symptoms such as fever, cough, sore throat, shortness of breath, and myalgia that can advance to respiratory failure and septic shock. Approximately 20% of cases have presented as asymptomatic or very mildly symptomatic; it is unknown if asymptomatic cases are contagious.[117]

4.2.6.4 Mortality

The case-fatality rate of MERS is estimated at almost 40%.[118] There is a clear positive correlation between increasing age and case-fatality rate. According to the WHO, the median age of MERS cases is 50 years old, with a range from nine months to 99 years.[119] Assiri et al. reported that among cases in Saudi Arabia, the case-fatality rate was 75% in patients over 60 years of age while there were no fatalities in patients younger than 19 years.[120]

Comorbidities also increase a patient's susceptibility to MERS-CoV. A large percent of MERS fatalities occur in patients with underlying medical conditions, such as diabetes and hypertension as well as chronic renal, lung, and cardiac disease.[121]

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110 Cowling BJ *et al* (2015) Preliminary epidemiologic assessment of MERS-CoV outbreak in South Korea, May–June 2015. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 20: 21163

111 Assiri A *et al* (2013b) Hospital outbreak of Middle East respiratory syndrome coronavirus. *N Engl J Med* 369: 407-416

112 Park HY *et al* (2015) Epidemiological investigation of MERS-CoV spread in a single hospital in South Korea, May to June 2015. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 20: 1-6

113 Cowling BJ *et al* (2015) Preliminary epidemiologic assessment of MERS-CoV outbreak in South Korea, May–June 2015. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 20: 21163

114 The World Health Organization (2014b) Middle East respiratory syndrome coronavirus (MERS-CoV). *WHO Risk Assesment* April 2014: 1-4

115 European Centre for Disease Prevention and Control (2015) Severe respiratory disease associated with Middle East respiratory syndrome coronavirus (MERS-CoV). *Rapid Risk Assessment* 20th update: 1-15

116 Memish ZA *et al* (2014) Middle East respiratory syndrome coronavirus (MERS-CoV) viral shedding in the respiratory tract: an observational analysis with infection control implications. *Int J Infect Dis* 29: 307-308

117 The World Health Organization (2015c) Management of asymptomatic persons who are RTPCR positive for Middle East respiratory syndrome coronavirus (MERS-CoV). *Interim guidance* July 2015: 1-3

118 Hussain HY (2014) Incidence and Mortality Rate of "Middle East Respiratory Syndrome"-Corona Virus (MERS-Cov), Threatens and Opportunities. *J Mycobac Dis* 5.

119 The World Health Organization (2015b) Summary of Current Situation, Literature Update and Risk Assessment. *Middle East respiratory syndrome coronavirus (MERS-CoV)* 15: 1-7

120 Assiri A *et al* (2013a) Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *The Lancet Infectious diseases* 13: 752-761

121 ibid.

4.3 An Overview of GoF Research

This section provides an overview of all Gain of Function (GoF) experimental approaches that are regularly used in the fields of coronavirus and influenza virus research. Our definition of "Gain of Function" includes all experimental approaches that are reasonably anticipated to lead to one or more of the following phenotypic changes, as defined in the NSABB's "Framework for Conducting Risk and Benefit Assessments of Gain of Function Research":

- Enhanced pathogen production as a result of changes in the replication cycle or growth,
- Enhanced morbidity and mortality in appropriate animal models,
- Enhanced transmission in mammals (e.g., increased host or tissue range, altered route of transmission, infectivity above a certain threshold determined in an appropriate animal model),
- Evasion of existing natural or induced immunity, and
- Resistance to drugs or evasion of other medical countermeasures such as vaccines, therapeutics, diagnostics.

These findings are based on two data sources: (1) a comprehensive review of the scientific literature involving influenza viruses and coronaviruses and (2) interviews with influenza virus and coronavirus researchers. Within the field of coronavirus research, our literature review included studies involving:

- SARS-CoV,
- MERS-CoV, and
- SARS- or MERS-like animal CoVs, including bat CoVs and civet CoVs.

We did not examine the scientific literature involving the four human coronaviruses that cause mild to moderate respiratory illnesses such as the common cold or croup: coronaviruses HKU1, OC43, 229E, and NL63. We note that throughout this report, our use of the term "coronaviruses" or "CoVs" refers specifically to SARS-CoV, MERS-CoV, and SARS/MERS-like animal CoVs such as HKU4 and HKU5. We identified approaches involving coronaviruses that are reasonably anticipated to lead to the following phenotypic changes:

- Enhanced pathogen production as a result of changes in the replication cycle or growth,
- Enhanced morbidity and mortality in appropriate animal models,
- Altered host range, and
- Evasion of therapeutics in development.

As current animal models for studying coronaviruses do not support transmission between animals, this field does not include any approaches that lead to enhanced transmission in appropriate animal models. Additionally, because there is no widespread population immunity to the coronaviruses and there are no licensed coronavirus vaccines, this field does not include any approaches that lead to evasion of existing natural or induced immunity. Finally, we did not identify any coronavirus research that is reasonably anticipated to lead to evasion of diagnostics or of vaccines in development. (We note that there are currently no FDA-approved vaccines or therapeutics for coronaviruses.)

Within the field of influenza research, our literature review included studies involving:

- Human seasonal strains: currently circulating and historical influenza A H1N1 and H3N2 viruses and influenza B viruses,
- Human pandemic strains: the 1918 H1N1, 1957 H2N2, 1968 H3N2, and 2009 H1N1 viruses,
- Swine-origin strains: H3N2v and others, and
- Avian-origin strains: H5N1, H7N9, H9N2 and others.

We identified approaches involving influenza viruses that are reasonably anticipated to lead to the following phenotypic changes:

- Enhanced pathogen production as a result of changes in the replication cycle or growth,
- Enhanced morbidity and mortality in appropriate animal models,
- Altered host range,
- Enhanced transmission in mammals,
- Evasion of existing natural or induced immunity,
- Evasion of therapeutics, and
- Evasion of vaccines in development.

We note that we are using the term "therapeutics" to include drugs that directly target viruses (e.g., influenza neuraminidase inhibitors), monoclonal antibody-based therapeutics, host immune modulators, and any other type of antiviral therapeutic. We did not identify any influenza research that is reasonably anticipated to lead to evasion of diagnostics.

We note that passaging of influenza viruses and coronaviruses in cells is essential for any experimental work involving live viruses, both to prepare virus stocks for experimental use and to conduct infection experiments. This applies to alt-GoF approaches, such as characterization of wild type viruses, as well as to GoF approaches. Because of the high mutation rates of RNA viruses, including influenza viruses and coronaviruses, such passaging inevitably selects for higher-yield viruses.[122] However, within the "enhanced virus production" phenotypic category, this analysis is restricted to those approaches that deliberately seek to enhance virus production through serial passaging, targeted genetic modification, or other approaches.

Below we briefly summarize the experimental approaches we identified within each phenotypic category, describing the experimental manipulation, virus strains that are used, and the scientific outcomes of each approach.

4.3.1 Coronaviruses

4.3.1.1 Enhanced Pathogen Production as a Result of Changes in the Replication Cycle or Growth

Serial passaging of CoVs in cell culture leads to the generation of higher-yield viruses. This approach has been performed using low-yield bat CoV strains to generate higher-yield strains that are suitable for experimental use. As SARS and MERS naturally grow well in the standard cell culture systems that are used in the field, researchers are not serially passaging either virus in cell culture to enhance virus production.

[122] Parvin JD *et al* (1986b) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. *Journal of virology* 59: 377-383

4.3.1.2 Altered Host Range

Several experimental approaches alter the host range of CoVs. One approach involves "Spike swapping," which is targeted genetic modification to replace all or part of the coronavirus Spike protein, a viral surface protein that mediates virus entry into cells and is a critical determinant of host restriction, with the Spike protein from another CoV species. This manipulation leads to the generation of a recombinant, chimeric CoV that may exhibit altered host tropism relative to the parental CoV species. The purpose of these experiments is three-fold:

- Introducing the SARS Spike protein into the backbone of bat CoVs, which do not efficiently infect standard cell culture lines or animals, enables the chimeric virus to infect cells/animals, thus creating a tool that can be used to study the biology of the bat CoV,
- Chimeric viruses are used as tools to test whether CoV therapeutics and vaccines are broad-spectrum, capable of protecting against potentially emerging SARS/MERS-like bat CoVs as well as SARS and MERS, and
- Testing the ability of chimeric CoVs to infect various types of cells and animals reveals the breadth of host tropism conferred by a given Spike protein, and comparing the sequences of parental and donated Spike proteins with different host tropism can uncover amino acid residues that mediate host restriction.

A second approach involves serial passaging of CoVs in mice, which leads to the generation of viruses that have adapted to more efficiently infect and cause disease in mice. The purpose of this experiment is two-fold:

- Mouse-adapted strains are experimental tools that are used for the study of disease pathogenesis and for testing the efficacy and safety of vaccines and therapeutics, and
- Comparing the sequences of the mouse-adapted and the parental strain leads to the identification of mutations that are associated with adaptation, which provides a foundation for follow-up studies investigating the mechanistic basis of virus adaptation to new hosts.

SARS CoV has been passaged in mice by multiple research groups to generate several different mouse-adapted strains; chimeric bat-SARS CoVs have also been passaged in mice. Serial passaging of MERS virus in mice, in order to generate a mouse model for the study of MERS, is ongoing.

A third approach involves targeted mutagenesis to introduce mutations that are associated with altered host tropism, which has been performed using SARS CoV. These mutations may have been discovered through a GoF approach, such as serial passaging, or through an alt-GoF approach, such as comparative sequence analysis. This experiment is performed to demonstrate that the mutation(s) are necessary and sufficient to alter host tropism, which provides a foundation for follow-up studies investigating the phenotypic traits underlying virus adaptation to new hosts.

4.3.1.3 Enhanced Morbidity and Mortality in Appropriate Animal Models

Several experimental approaches enhance the fitness or virulence of CoVs in cell culture or laboratory animal model systems, respectively. First, serial passaging of CoVs in mice leads to the generation of viruses with both enhanced infectivity to and virulence in mice. Because of the specificity of virus-host

interactions that are important determinants of host tropism and pathogenicity, this adaptation often translates to reduced virulence in humans. The purpose of this experiment is two-fold:

- Enhancing the virulence of the virus in mice is an important aspect of creating a mouse model that replicates human disease pathology, which is needed for the study of disease pathogenesis mechanisms and the testing of medical countermeasures, and
- Comparing the sequences of the mouse-adapted and the parental strain leads to the identification of mutations that are associated with enhanced virulence, which provides a foundation for follow-up studies to elucidate the mechanistic basis of virulence. This information can also benefit public health by identifying new potential targets for therapeutics or for attenuation, in order to create attenuated vaccine viruses.

A second approach involves targeted genetic modification of viruses to introduce mutations that are associated with enhanced virulence, which is performed to demonstrate that the mutation(s) are necessary and sufficient to enhance virulence. As above, this information provides a foundation for follow-up studies to elucidate the mechanistic basis of virulence.

A final approach involves serial passaging of attenuated viruses in cells or in animals, in order to determine whether viruses can recover fitness/virulence upon growth in appropriate model systems. This approach is performed using attenuated viruses that could be used as live attenuated vaccines (LAVs). Because LAVs with an ability to recover fitness during growth *in vivo* could cause adverse outcomes in people, a negative result is an important indicator of safety for any live attenuated vaccine in development.

4.3.1.4 Evasion of Therapeutics in Development

Serial passaging of a virus in cells in the presence of a therapeutic may lead to the emergence of viruses that are resistant to inhibition/neutralization by that therapeutic. This type of experiment has been performed using SARS CoV, in order to select for escape from monoclonal antibody therapeutics and other types of therapeutics. The purpose of the experiment is to understand whether and how readily resistance will arise in response to selective pressure from the therapeutic and to identify mutations that are associated with resistance to the therapeutic, which provides a foundation for follow-up studies investigating the mechanisms underlying antiviral activity and antiviral resistance. Because there are no FDA-approved therapeutics for CoVs, this approach has exclusively been applied to the study of therapeutics in development.

4.3.2 Influenza viruses

4.3.2.1 Enhanced Pathogen Production as a Result of Changes in the Replication Cycle or Growth

Several experimental approaches lead to enhanced production of influenza viruses. The first approach involves reassortment between a wild type strain and an attenuated, high-yield vaccine backbone strain to generate a "Candidate Vaccine Virus" (CVV), which comprises the HA and NA genes from the wild type strain and the remaining six "internal genes" from the vaccine backbone strain and exhibits higher levels of growth than the parental, wild type virus. CVVs are attenuated and exhibit higher levels of growth relative to the parental, wild type virus. CVVs may be generated through classical reassortment methods, which involve co-infection of eggs or cells with the wild type strain and the vaccine backbone strain followed by antibody-based selection for viruses with the correct surface antigens or through reverse

genetics.[123] These approaches are currently used for the production of influenza vaccines in eggs or cells – high-yield CVVs serve as the basis for the vaccine strains that are used by manufacturers for large-scale production. In addition, comparing the sequences of CVVs with different growth properties can lead to the identification of mutations associated with high growth.

The second approach involves serial passaging of viruses in cells, which selects for higher-yield viruses. This approach is also a core aspect of the current production of influenza vaccines in eggs or cells. Specifically, manufacturers serially passage CVVs in eggs or cells to increase CVV yields and to optimize growth and infection conditions in order to create a vaccine seed strain that is used for large-scale production of vaccine viruses. The serial passaging approach is also used in academic research, primarily involving vaccine backbone strains and CVVs but occasionally involving wild type viruses. In addition to supporting vaccine development, the goals of this experiment could be to identify mutations associated with high yield, which provides a foundation for follow-up studies investigating the mechanistic basis of high growth in cells or eggs.

Third, forward genetic screens, which involve random mutagenesis of viruses followed by limited passaging to select for mutants with high growth properties, enable the identification of mutations that confer high growth to viruses. Forward genetic screens involving vaccine backbone strains and CVVs lead to the identification of mutations that are sufficient to enhance the yields of vaccine viruses.

A final approach involves targeted mutagenesis of viruses to introduce mutations that are associated with high growth. These mutations may have been discovered through a GoF approach, such as serial passaging or a forward genetic screen, or through an alt-GoF approach, such as comparative analysis of wild type sequences. This experiment is performed to demonstrate that the mutation(s) are necessary and sufficient to enhance virus production, which provides a foundation for follow-up studies investigating the mechanistic basis of the high-growth phenotype.

We note that experimental approaches involving targeted genetic modification of the viral polymerase complex of avian viruses to render it more "human-like" (through site-directed mutagenesis or reassortment between human and avian viruses) is also likely to enhance virus replication. However, as the primary goal of those studies is to gain insight into the mechanisms underlying adaptation of avian viruses to mammals, we discuss those studies in the "enhanced transmission in mammals" section.

4.3.2.2 Altered Host Range

Several experimental approaches lead to the generation of viruses with altered host range. First, serial passaging of viruses in mammalian cells or tissues or in laboratory animals selects for viruses with enhanced growth in cells or enhanced infectivity to animals, respectively. This type of serial passaging experiment involves "forced" passaging, meaning that the experimenter directly transfers infected material, in the form of cell culture supernatant or homogenates of infected tissue, to the subsequent cell culture dish or animal. Forced serial passaging is carried out for two purposes: (1) to identify mutations that arise during adaptation of animal-origin viruses (i.e., avian and swine viruses) to mammals, which provides a foundation for follow-up studies investigating the evolutionary mechanisms driving adaptation to mammalian hosts and the mechanistic basis of mammalian adaptation, and (2) to develop an mouse model for the study of a particular virus. Avian and swine viruses are used for studies that seek to understand the mechanisms underlying mammalian adaptation, and human seasonal viruses are primarily used for studies that aim to generate new mouse models.

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[123] Use of classical reassortment methods to generate CVVs may lead to the generation of a 5:3 reassortment strain which includes the HA, NA, and one additional gene from the wild type strain and the remaining five genes from the vaccine backbone strain.

A second approach involves deliberate genetic modification of viruses, namely site-directed mutagenesis and/or reassortment, to introduce genetic traits that may enhance fitness/infectivity in mammals. These mutations or reassortment gene combinations may be random (i.e., for a forward genetic screen) or may have been previously shown to be associated with mammalian adaptation or an underlying phenotype, such as a preference for host receptors decorated with 'human-like' sialic acid moieties. Notably, genetic traits that are associated with mammalian adaptation may be discovered through GoF approaches, such as serial passaging, or alt-GoF approaches, such as comparative sequence analysis of avian viruses isolated from human versus poultry infections. Collectively, the deliberate genetic modification approach is used to discover new genetic traits that contribute to mammalian adaptation and to confirm that particular genetic traits are necessary and sufficient to enhance fitness/infectivity in mammals. Animal viruses, including avian and swine viruses, are used exclusively for these studies.

4.3.2.3 Enhanced Transmission in Mammals

Several experimental approaches lead to the generation of viruses with enhanced transmissibility in mammals. First, serial passaging of viruses in animals with selection for transmission leads to the generation of viruses with enhanced transmissibility in mammals. This type of serial passaging experiment can involve selection for contact transmission, during which the primary (directly inoculated) and secondary hosts are co-housed, or for airborne transmission, during which the primary and secondary hosts are separately housed in special isolator cages that prevent direct contact between animals but allow for air exchange between cages. These studies seek to identify mutations that are sufficient to enhance transmissibility, which provides a foundation for follow-up studies that investigate the mechanistic basis of transmissibility in mammals. Animal viruses, including avian and swine viruses, are used exclusively for these studies.

A second approach involves deliberate genetic modification of viruses, namely site-directed mutagenesis and/or reassortment, to introduce genetic traits that may enhance transmissibility in mammals. These mutations or reassortment gene combinations may be random (i.e., for a forward genetic screen) or may have been previously shown to be associated with transmissibility or an underlying phenotype, such as an increase in the stability of the HA protein. Notably, genetic traits that are associated with transmissibility may be discovered through GoF approaches, such as serial passaging, or alt-GoF approaches, such as forward genetic screens to identify mutations that alter HA stability performed using *in vitro*, virus-free systems. Collectively, the deliberate genetic modification approach is used to discover new genetic traits that contribute to transmissibility and to confirm that particular genetic traits are necessary and sufficient to enhance transmissibility in mammals. Animal viruses, including avian and swine viruses, are used exclusively for these studies.

4.3.2.4 Enhanced Morbidity and Mortality in Appropriate Animal Models

Akin to the enhanced transmission phenotype, both serial passaging and deliberate genetic modification approaches can lead to the generation of viruses with enhanced morbidity and mortality in appropriate animal models. Serial passaging of viruses in animals selects for viruses with enhanced virulence and is used for three purposes. First, serial passaging is utilized to develop animal models for studying the mechanistic basis of flu-associated morbidity/mortality and for medical countermeasure development (as adapting a virus to an animal typically enhances its virulence in that host). Second, this approach enables the identification of mutations that are associated with enhanced fitness/virulence, which provides a foundation for follow-up studies that investigate the mechanistic basis of pathogenicity. These studies can also provide insight into host mechanisms underlying disease pathology by correlating host immune responses with morbidity and mortality measures. Third, the serial passaging approach is used to determine whether attenuated strains are capable of recovering virulence upon passage *in vitro* or *in vivo*.

This third type of serial passaging study may be carried out using live attenuated influenza vaccine (LAIV) candidates, as an important aspect of safety testing prior to human clinical trials. In addition, these studies may be conducted using strains with fitness defects arising from the acquisition of antiviral resistance or other GoF phenotypes, in order to gain insight into the likelihood that these strains will persist and spread in nature. All types of serial passaging studies may be performed with seasonal or animal (i.e., avian and swine) viruses, and animals such as mice, ferrets, and swine may be used. Of note, serial passaging studies involving attenuated strains simply increase the human health risk of the attenuated strain to approach that of wild type strains.

A second approach involves deliberate genetic modification of viruses, through either site-directed mutagenesis or reassortment, to introduce genetic traits that are expected to enhance pathogenicity. As above, these mutations or reassortment gene combinations may be random (e.g., for a forward genetic screen) or may have been previously shown to be associated with a phenotype underlying pathogenicity, such as evasion of a particular innate immune response. Traits that are associated with enhanced pathogenicity may be discovered through GoF approaches, such as serial passaging, or alt-GoF approaches, such as random mutagenesis followed by screening for attenuated virulence (Loss of Function). Collectively, the deliberate genetic modification approach is used to discover new genetic traits that contribute to pathogenicity and to confirm that particular genetic traits are necessary and sufficient to enhance virulence in mammals. These studies are performed using human seasonal viruses, the 1918 H1N1 pandemic virus, and animal viruses.

We note that the relationship between viral fitness and pathogenicity is complex and that many of the viral traits that contribute to fitness, either directly or indirectly, mediate pathogenicity. As a result, serial passaging of viruses in animals may select for both enhanced fitness and enhanced virulence. However, enhanced viral fitness *in vivo* does not necessarily translate to high pathogenicity, as seasonal influenza viruses do not display the morbidity and mortality displayed during infections with zoonotic influenza viruses such as H5N1, but grow to a high titer.

4.3.2.5 Evasion of Existing Natural or Induced Adaptive Immunity

Several experimental approaches can lead to the generation of viruses that evade existing natural or induced immunity. First, serial passaging of viruses in the presence of cognate antibodies may lead to the acquisition of mutations that allow the virus to escape neutralization by the antibody. This experiment can be performed in cell culture or in animals that have been vaccinated or previously exposed to influenza viruses. The second approach involves deliberate modification of the influenza HA protein, the immunodominant influenza protein and the primary component of influenza vaccine, to introduce mutations that may lead to antigenic change. In this case, the mutations may be random (i.e., in the context of a forward genetic screen), previously identified through a GoF approach such as serial passaging, or previously identified through an alt-GoF approach such as comparative analysis of wild type sequences. When either approach is performed using recently or currently circulating seasonal influenza viruses or using seasonal viruses that have recently served as the basis for vaccine strains, the end result is the generation of a mutant strain that cannot be neutralized by existing natural or induced immunity, respectively. These studies aim to identify amino acid substitutions that lead to antigenic change and to define the evolutionary pathways by which those substitutions arise, which provides a foundation for follow-up studies investigating the evolutionary mechanisms driving antigenic drift and the molecular basis of antigenic differences between strains.

Because human populations do not have widespread immunity to the 1918 H1N1 pandemic virus or to animal influenza viruses (i.e., avian viruses and swine viruses), no approaches involving these viruses meet this phenotypic criterion.

4.3.2.6 Evasion of Vaccines in Development

Serial passaging of a virus in cells in the presence of animal sera produced in response to a candidate vaccine or in animals vaccinated with a candidate vaccine may lead to the emergence of viruses that are resistant to neutralization by that vaccine. This approach is used to test whether and how readily viruses can evolve to evade vaccines in development, for example new vaccine platforms that are more broad-spectrum or resistant to drift than current influenza vaccine platforms, which is an important indicator of the potential field efficacy of the vaccine. Most of these experiments involve next-generation influenza vaccine candidates targeting epitopes other than the globular head domain of the hemagglutinin (HA) protein, the target of current influenza vaccines. Given that the globular head domain of HA is the immunodominant protein of influenza viruses and that these next-generation vaccines are not yet widely available, strains that can overcome the protection afforded by these vaccines are expected to pose a minimal increase in human health risk relative to wild type strains.

Because seasonal influenza vaccines are updated annually, approaches that lead to the generation of vaccine strains that are no longer neutralized by vaccine-induced antibodies are more appropriately described by the "evasion of existing induced immunity" phenotype. In addition, we did not identify any studies involving H5N1 viruses that would be expected to lead to the generation of viruses that cannot be neutralized by the pre-pandemic H5N1 vaccine in the national stockpile.

4.3.2.7 Evasion of Therapeutics

Several approaches may lead to the generation of viruses that are resistant to therapeutics. The classical approach involves serial passaging of viruses in the presence of a therapeutic, which may lead to the acquisition of mutations that allow the virus to evade inhibition by the therapeutic. This approach is performed to determine whether and how readily a virus evolves resistance in response to selective pressure from a therapeutic and to identify mutations that confer resistance, which provides a foundation for follow-up studies investigating the mechanism of action of the therapeutic and the mechanistic basis of antiviral resistance. When passaging experiments are performed using a new therapeutic candidate with an unknown viral target, this information also helps to identify the therapeutic target, as resistance mutations are most likely to arise in the target protein. Of note, the acquisition of resistance to novel classes of therapeutics is not expected to confer cross-resistance to existing antivirals (i.e., adamantanes or neuraminidase inhibitors). Thus, when these experiments involve drug candidates within new classes of therapeutics, which are not yet widely available, no increase in human health risk is posed by resistant strains. Serial passaging approaches have been performed using cell culture, animal models, and (rarely) human challenge experiments.

The second approach involves deliberate modification of antiviral target proteins to introduce mutations that may confer antiviral resistance. In this case, the mutations may be random (i.e., in the context of a forward genetic screen), previously identified through a GoF approach such as serial passaging, or previously identified through an alt-GoF approach such as comparative analysis of wild type sequences. Similar to serial passaging experiments, these experiments provide a foundation for follow-up studies investigating the mechanistic basis of antiviral resistance. Both types of GoF approaches have been performed using human seasonal viruses, human pandemic strains (i.e., the 1957 H2N2 pandemic virus), and animal-origin strains. (We note that human challenge experiments have only been performed using human seasonal strains.)

4.3.2.8 Reassortment

Several experimental approaches can be used to assess the genetic compatibility and fitness of viruses following reassortment. While the phenotypic consequences of reassortment events between two viruses

cannot be predicted with certainty, reassortant strains may exhibit enhanced fitness, pathogenicity, and/or transmissibility relative to one or both parental strains. (Notably, reassortant strains may also display *reduced* fitness, pathogenicity, and/or transmissibility relative to parental viruses.) In the laboratory, reassortant viruses can be generated through reverse genetics, which involves the deliberate mixing of gene segments from two or more viruses in one or multiple combinations or through co-infection of cells or animals with two viruses. Follow-up studies may be performed to evaluate pathogenicity, infectivity, and/or transmissibility of viable reassortants. Collectively, these approaches assess the viability and phenotypic properties of various reassortment viruses. This information provides a foundation for studies investigating mechanisms governing reassortment and informs the potential for reassortant viruses to emerge in nature and the potential public health consequences of such an emergence event.

5 Historical Context of Outbreaks of Influenza, SARS and MERS

5.1 Purpose and Context

In this section, we review the history and current status of influenza, SARS, and MERS to provide context for evaluating the potential risks and benefits associated with GOF studies. Naturally-occurring epidemics and pandemics present risks to human and animal health and burdens to public health infrastructure. Such risks are pertinent to the ongoing deliberative process on the risks and benefits of GOF research as they help to establish the existing risks associated with infectious diseases to which the risks associated with GOF studies might be compared. This historical perspective will also inform evaluations of the potential benefit of interventions to reduce the burden of these diseases on society; the greater the harm that these diseases have inflicted, the greater the potential benefit to society of mitigating their harm.

To the extent available, we have gathered data on past outbreaks of these diseases and the morbidity, mortality, and economic harm that they have inflicted. For seasonal influenza, these data should provide a solid baseline for understanding the potential benefits of reducing the burden of this disease that continues to afflict public health annually. Caution should be used when reviewing the data on outbreaks of pandemic influenza strains and the human coronaviruses because the disease caused by each new strain has unique attributes that influence its extent and severity. The next outbreak from a newly emergent influenza virus or coronavirus could be as severe, not nearly as severe as, or more severe than any of the historical outbreaks and there is no science-based means to determine the severity a priori.

For example, although the 1918 influenza outbreak is often held up as the exemplar of the type of pandemic that researchers are trying to prevent, detect early, or mitigate, the severity and extent of the outbreak may be only partially explained by its unique biology. This pandemic occurred in the waning years of a world war, when the nutritional status and overall health of the global population was compromised and when living conditions for the most vulnerable populations were poor (in this case, younger adults). Perhaps more importantly, public health systems (which rely on the public's understanding of the seriousness of infectious disease threats) are far more robust today, and therefore today's social distancing measures and mass vaccination may greatly mitigate the consequences of an outbreak. Conversely, the society of the early 20th century was less reliant on complex networks to provide food, security, water, and sanitation services, which could crumble in the face of an outbreak of a disease that kills a significant number of otherwise healthy adults, leading to secondary deaths from starvation or social disorder.

Note that, although the findings in this section provide historical background and context for the viruses studied, they do not directly provide the epidemiological parameters used in the biosafety risk assessment described in Chapter 6. Because the biosafety risk assessment was done parametrically, for each virus a range of values was used for each of the parameters. In certain cases, the values from historical outbreaks described here are used to provide an upper or lower bound for an epidemiological parameter. However, in general the ranges of values used in the biosafety risk assessment are broader, to account for the epistemic uncertainty in some of the values, to encompass all potentially possible naturally occurring strains, and to encompass the range of gain-of-function modifications that may be done to them. Further information on the range of values used in each of the biosafety models is described in the Supporting Information section for each model.

5.2 Severe Acute Respiratory Syndrome

5.2.1 Summary of Findings

- In 2003, an outbreak of SARS occurred in several Asian countries and Canada, causing nearly 10,000 illnesses and 1,000 deaths, with a disproportionate burden on the elderly,
- Most survivors suffer from long term physical and mental morbidities,
- The outbreak was responsible for $30-100Bn in economic losses, and
- No human cases of SARS have been reported since 2004.

5.2.2 Background

Severe acute respiratory syndrome (SARS) is a viral respiratory disease of zoonotic origin, caused by a coronavirus identified as SARS-associated coronavirus, or SARS-CoV. Despite ample research, the natural reservoir of SARS-CoV has not been documented conclusively. The Himalayan masked palm civet (*Paguma larvata*), a delicacy in southern China, is commonly attributed as the human transmission source, but other Chinese delicacies, such as ferret badger (*Melogalemoschata*), as well as domestic cats (*Felis domesticus)*, ferrets (*Mustela putorius furo*), and bats (*Rhinolophus)* have also been laboratory-confirmed as virus reservoirs.[124]

SARS typically begins with flu-like symptoms, including high fever, fatigue, sore throat, headache, and myalgia. Some patients also experience diarrhea, dry cough, and shortness of breath. As SARS progresses, most cases will develop into pneumonia. The disease spreads through close contact between people, mainly by droplet spread of infectious fluids.[125] Without treatment, the pneumonia may lead to respiratory failure and death.

5.2.3 Initiation of the SARS Outbreak

In November 2002, atypical pneumonia of an unknown cause was reported in two patients in Fushan City, southern Guangdong Province, China. Soon after, similar cases were reported in five other Guangdong cities.[126] The virus remained in China until February 2003 when a physician who had been treating SARS patients traveled from Guangdong to a hotel in Hong Kong. There he infected ten others from various countries who then continued traveling, bringing the virus with them to Ireland, Canada, Vietnam, Singapore, and the United States (Figure 5.1).[127]

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[124] The World Health Organization. Severe acute respiratory syndrome. http://www.who.int/topics/sars/en/. Last Update Accessed July 2015.
[125] The Centers for Disease Control and Prevention (2004) Basic Information about SARS *Fact Sheet*
[126] Wang MD, Jolly AM (2004) Changing virulence of the SARS virus: the epidemiological evidence. *Bull World Health Organ* 82: 547-548
[127] Christian MD *et al* (2004) Severe acute respiratory syndrome. *Clin Infect Dis* 38: 1420-1427

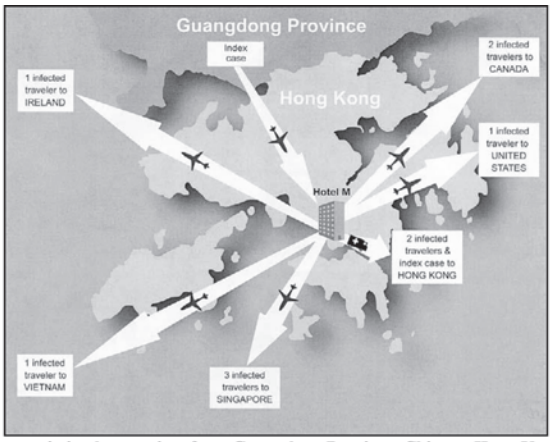

Figure 5.1. SARS transmission by a patient from Guangdong Province, China to Hong Kong and then to global travelers reproduced from Christian MD et al.[128]

These cases sparked a global outbreak. Within weeks, the communicable illness spread to 37 countries around the world and became recognized as the SARS epidemic of 2003.[129] Figure 5.2 below shows the explosiveness of the outbreak after the international transmission began.[130]

[128] ibid.
[129] Wang MD, Jolly AM (2004) Changing virulence of the SARS virus: the epidemiological evidence. *Bull World Health Organ* 82: 547-548
[130] Braden CR *et al* (2013) Progress in global surveillance and response capacity 10 years after severe acute respiratory syndrome. *Emerg Infect Dis* 19: 864-869

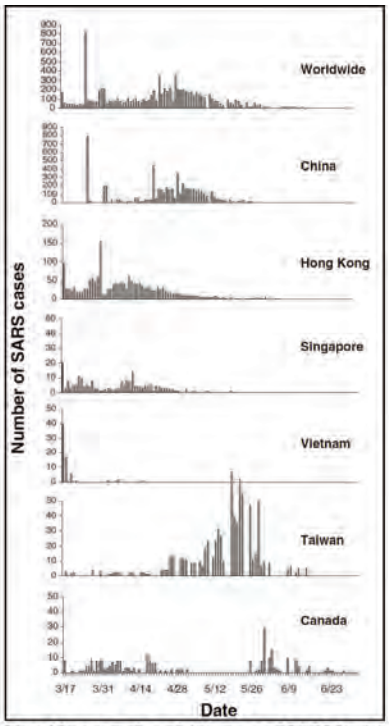

Figure 5.2. Reproduced from Chan-Yeung et. al, probable cases of SARS by date of onset or reporting worldwide.[131]

SARS is known to transmit through close person-to-person contact and droplet spread, however large, localized outbreaks suggest additional methods of transmission. Hong Kong's index case can be traced to an outbreak in a large apartment complex, Amoy Gardens, where 329 residents became infected.[132] The index case did not contact all three-hundred residents which, with the ability of SARS to remain viable in feces, presents the possibility of fecal-droplet transmission through the plumbing system of the apartment complex.[133]

The CDC also investigated "super spreaders" which are believed to be highly infectious index cases such as the Guangdong doctor that traveled to a hotel in Hong Kong and the resultant rapid outbreak in Canada. Possible explanations included a higher SARS-CoV infectious load, aerosolized transmission that allowed the particles to travel further, and increased age or previous illness that masked the SARS

131 Chan-Yeung M, Xu R-H (2003) SARS: epidemiology. *Respirology* 8: S9-S14
132 Ibid.
133 Christian MD *et al* (2004) Severe acute respiratory syndrome. *Clin Infect Dis* 38: 1420-1427

infection. Rapid transmission by super spreaders is credited with initiation and continuation of the SARS outbreak.[134]

5.2.4 Morbidity and Morality

According to the World Health Organization (WHO), there were 8,098 SARS cases reported worldwide during the 2003 epidemic, which resulted in 744 deaths.[135] Approximately 30% of cases are believed to have occurred in healthcare workers due to the necessity of close contact in transmission.[136] In the United States, there were only eight laboratory-confirmed cases and no SARS-associated deaths. All eight patients had traveled to SARS-infected regions, but did not further transmit the disease upon returning to the US.[137]

The WHO estimates the overall case fatality rate of the SARS outbreak to be 11%, with a range of 0%-50% among age groups.[138] Another study estimates the range to be between 0%- 40% with an overall fatality rate of 9.6%.[139]

The overall fatality rate, however, minimizes the significant difference that occurred between age groups. SARS disproportionately kills older people and although the incidence rates did not differ amongst age groups, the mortality rate in those over 75 years old was 38% whereas no deaths occurred in those under 24 years.[140] Advanced age is the most influential risk factor for SARS-associated death. One study determined the case fatality rate for persons under 60 years old to be 6.8% while the rate for over 60 years was 55%.[141] Another study estimated the average case fatality rate at 45% for persons over 60 years.[142] These statistics are provided in Table 5.1 below. All affected regions experienced similar age-specific trends with a large variance between groups, as can be seen in Figure 5.3 from Anderson et al.

Table 5.1. SARS Case Fatality Rates Among Age Groups

Source	Overall	< 24 years	< 60 years	> 60 years	> 75 years
Christian et al. 2004[143]	9.6%	-	-	45%	-
Wang et al. 2004[144]	-	0%	-	-	38%
Donnelly et al. 2003[145]	-	-	6.8%	55%	-

[134] Centers for Disease Control and Prevention. Remembering SARS: A Deadly Puzzle and the Efforts to Solve It. Last Update April 2013. Accessed

[135] Guan Y *et al* Molecular epidemiology of the novel coronavirus that causes severe acute respiratory syndrome. *The Lancet* 363: 99-104

[136] The World Health Organization (2003b) Severe acute respiratory syndrome (SARS): Status of the outbreak and lessons for the immediate future. *Unmasking a new disease*

[137] The Centers for Disease Control and Prevention (2004) Basic Information about SARS *Fact Sheet*

[138] World Health Organization. Alert, verification and public health management of SARS in the post-outbreak period. http://www.who.int/csr/sars/postoutbreak/en/. Last Update August 14, 2003. Accessed July 2015.

[139] Christian MD *et al* (2004) Severe acute respiratory syndrome. *Clin Infect Dis* 38: 1420-1427

[140] Wang MD, Jolly AM (2004) Changing virulence of the SARS virus: the epidemiological evidence. *Bull World Health Organ* 82: 547-548

[141] Donnelly CA *et al* (2003) Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* 361: 1761-1766

[142] Christian MD *et al* (2004) Severe acute respiratory syndrome. *Clin Infect Dis* 38: 1420-1427

[143] Ibid.

[144] Wang MD, Jolly AM (2004) Changing virulence of the SARS virus: the epidemiological evidence. *Bull World Health Organ* 82: 547-548

[145] Donnelly CA *et al* (2003) Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *Lancet* 361: 1761-1766

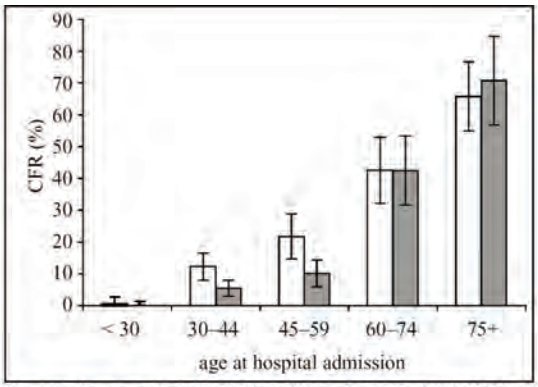

Figure 5.3. Case fatality rates by age and gender in Hong Kong during the 2003 SARS epidemic. Figure reproduced from Anderson et. al (white bars: male; grey bars: females).[146]

Fatality rates also varied between geographical regions. Table 5.2 below, from Chan-Yueng et al., displays the number of cases and deaths as well as case fatality rate by country.

Table 5.2. The Cumulative Number of Cases, Number of Deaths, and Case Fatality Rate by Country During the 2003 SARS Epidemic. Table Reproduced from Chan-Yeung et.al [147]

Country	Cumulative number of cases	Number of Deaths	Case-fatality rate (%)
Australia	5	0	-
Canada	251	41	17
China	5327	349	7
Hong Kong SAR, China	1755	300	17
Taiwan	346	37	11
Indonesia	2	0	-
Malaysia	5	2	-
New Zealand	1	0	-
Philippines	14	2	-
Korea	3	0	-
Singapore	238	33	14
Thailand	9	2	-
Vietnam	63	2	8
Global	**8098**	**774**	**9.6**

[146] Anderson RM *et al* (2004) Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* 359: 1091-1105.
[147] Chan-Yeung M, Xu R-H (2003) SARS: epidemiology. *Respirology* 8: S9-S14

Overall mortality rates varied from 0% to 17.1% by region. As the epidemic proceeded, these rates began to climb. Regions affected early by SARS, such as Guangdong, had case fatality rates ranging from 4% to 10%; regions affected later in the outbreak experienced higher rates, upwards of 13% to 17%.[148] One explanation for this trend is a younger population in regions affected earlier by the virus. Another possibility is evolution towards a more virulent strain, providing the virus with greater opportunities for transmission. Worsened symptoms increase the likelihood of droplet spread as well as the need for more medical treatment and hence, risk of human transmission.[149]

5.2.5 Long-Term Morbidity

SARS survivors experienced significant long-term morbidity. Mak et al. used the MOS 36-Item Short Form (SF-36), a functional outcome assessment, to measure the quality of life of survivors post-illness in Hong Kong. The study found that SARS survivors performed poorer in all eight quality of life categories than the normal population (Table 5.3).[150] In 2009, the Chinese media reported that 300 survivors declared continued complications from their illness. Approximately 60% still suffered from medical issues, including avascular necrosis and pulmonary fibrosis, and 80% could no longer work. Chronic depression was also reported.[151]

Table 5.3. Quality of Life Based on Eight Domains of the SF-36 Assessment Between SARS Survivors and the Normal Population 30 Months After the Sars Epidemic. Table Reproduced from Mak et. al[152]

Quality of Life	SARS Subjects (n=90)	HK Population normative values	P[a]
Physical functioning	75.17±22.77	91.83±12.89	<.001**
Role limitations due to physical health	43.54±46.39	82.43±30.97	<.001**
Bodily pain	58.74±29.98	83.98±21.89	<.001**
General health	40.18±26.58	55.98±20.18	<.001**
Vitality	48.82±22.32	60.27±18.65	<.001**
Social functioning	67.07±27.81	91.19±16.57	<.001**
Role limitations dues to emotional health	51.70±46.35	71.66±38.36	<.001**
Mental health	61.62±21.57	72.79±16.57	<.001**

Values are mean ± S.D.

[a] Two-sided independent sample t test.

*** P<.001*

148 Wang MD, Jolly AM (2004) Changing virulence of the SARS virus: the epidemiological evidence. *Bull World Health Organ* 82: 547-548

149 Ibid.

150 Mak IWC *et al* Long-term psychiatric morbidities among SARS survivors. *General Hospital Psychiatry* 31: 318-326

151 Xiang YT *et al* (2014) Outcomes of SARS survivors in China: not only physical and psychiatric co-morbidities. *East Asian archives of psychiatry : official journal of the Hong Kong College of Psychiatrists = Dong Ya jing shen ke xue zhi : Xianggang jing shen ke yi xue yuan qi kan* 24: 37-38

152 Mak IWC *et al* Long-term psychiatric morbidities among SARS survivors. *General Hospital Psychiatry* 31: 318-326

Many SARS survivors suffer from mental morbidity. Several studies have examined the psychiatric status of survivors at different time points, populations, and locations. Because of these differences, the percentage of survivors with a psychiatric disorder has fluctuated, however, the burden of mental illness (Table 5.4). Health care workers also tended to have higher stress levels and depressive symptoms than non-workers, with 90% qualifying as a potential psychiatric case.[153]

Table 5.4. Percentage of the Measured Population with a Psychiatric Disorder After the SARS Epidemic

Source	Time After Epidemic	Psychiatric Disorder
Lee et al. 2007[154]	1 year	64%
Mak et al. 2009[155]	30 months	33.3%
Lam et al. 2009[156]	4 years	42.5%

5.2.6 Economic Burden

The SARS epidemic is estimated to have cost anywhere from $30 to $100 billion worldwide from treatment costs, productivity loss, and decrease in travel and tourism.[157] Some estimates place the economic burden at US $30 billion in the Far East alone.[158] This burden translated into a decrease in Gross Domestic Product (GDP) for many countries. Hong Kong experienced the greatest loss, with a 2.63% decline in GDP, while China's GDP fell 1.05%. GDP in United States fell 0.07%.[159] While the percentages may appear small, Table 5.5 shows the estimated economic loss in US dollars resulting from the epidemic.

153 Lee AM *et al* (2007) Stress and psychological distress among SARS survivors 1 year after the outbreak. *Canadian journal of psychiatry* 52: 233

154 Ibid.

155 Mak IWC *et al* Long-term psychiatric morbidities among SARS survivors. *General Hospital Psychiatry* 31: 318-326

156 Xiang YT *et al* (2014) Outcomes of SARS survivors in China: not only physical and psychiatric co-morbidities. *East Asian archives of psychiatry : official journal of the Hong Kong College of Psychiatrists = Dong Ya jing shen ke xue zhi : Xianggang jing shen ke yi xue yuan qi kan* 24: 37-38

157 Smith RD (2006) Responding to global infectious disease outbreaks: lessons from SARS on the role of risk perception, communication and management. *Soc Sci Med* 63: 3113-3123

158 The World Health Organization (2003b) Severe acute respiratory syndrome (SARS). Status of the outbreak and lessons for the immediate future. *Unmasking a new disease*

159 (2004) Estimating the Global Economic Cost of SARS. In *Learning from SARS: Preparing for the Next Disease Outbreak: Workshop Summary*, Knobler S, Mahmoud A, Lemon S, Mack A, Sivitz L, Oberholtzer K (eds). Washington (DC)

Table 5.5. Estimates of the Economic Consequences of the SARS Epidemic by Region and Worldwide (in USD)		
Source	**Region**	**Economic Loss**
Mackenzie et al. 2013[160]	Worldwide	$40 billion
Lee et al. 2004[161]	Worldwide	$40- $54 billion
Wen et al. 2004[162]	China	$25.3 billion
Siu et al. 2004[163]	China	$48 billion
Fan 2003[164]	East & Southeast Asia	$12.3- $28.4 billion

On May 18, 2004, the WHO declared the last SARS case to be contained. No human infections have been reported since due to the stringent disease control and public health response.[165]

5.3 Middle East Respiratory Syndrome

5.3.1 Summary

- Outbreaks of MERS were first identified in 2012 and have occurred sporadically since that time,
- To date these outbreaks have led to more than 1,000 cases and nearly 500 deaths, the burden of which fell disproportionately on the elderly with significant co-morbidities, and
- The vast majority of cases have been identified in the Middle East, and, recently South Korea although cases have been identified sporadically in countries in Europe and North America.

5.3.2 Background

Middle East Respiratory Syndrome (MERS) is an acute severe respiratory infection of zoonotic origin caused by a corona virus knows as MERS-CoV. Although the natural source is unconfirmed, MERS-CoV has been identified in camels in the Arabian Peninsula. MERS transmission has occurred through close contact between humans and animals, most likely dromedary camels or bats.[166] Both the MERS-CoV virus and its antibodies have been isolated in dromedary camels in the Arabian Peninsula. These findings reinforce the possibility of camel-to-human transmission through close contact and the ingestion of raw camel milk. While camel meat could also be a source of infection, cooking the meat is customary and inactivates the virus.[167]

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160 Mackenzie JS, Merianos A (2013) The legacies of SARS - international preparedness and readiness to respond to future threats in the Western Pacific Region. *Western Pacific surveillance and response journal : WPSAR* 4: 4-8

161 (2004) Estimating the Global Economic Cost of SARS. In *Learning from SARS: Preparing for the Next Disease Outbreak: Workshop Summary*, Knobler S, Mahmoud A, Lemon S, Mack A, Sivitz L, Oberholtzer K (eds). Washington (DC)

162 Wen H *et al* (2004) The Short-Term Impact of SARS on the Chinese Economy. *Asian Economic Papers* 3: 57-61

163 Siu A, Wong YCR ibid Economic Impact of SARS: The Case of Hong Kong. 62-83

164 Fan, Pruma Xiaoqin. 2003. *SARS: Economic Impacts and Implications*. © Asian Development Bank. http://hdl.handle.net/11540/616. License: CC BY 3.0 IGO.

165 Mackenzie JS, Merianos A (2013) The legacies of SARS - international preparedness and readiness to respond to future threats in the Western Pacific Region. *Western Pacific surveillance and response journal : WPSAR* 4: 4-8

166 Penttinen PM *et al* (2013) Taking stock of the first 133 MERS coronavirus cases globally--Is the epidemic changing? *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 18

167 Abdel-Moneim AS (2014) Middle East respiratory syndrome coronavirus (MERS-CoV): evidence and speculations. *Arch Virol* 159: 1575-1584

Patients display symptoms such as fever, cough, shortness of breath, sore throat, and myalgia; some infected people show no symptoms at all. Approximately three to four out of every ten persons suspected of MERS have died. Most fatalities, however, had significant co-morbidities that exacerbated the MERS symptoms.[168]

5.3.3 The Emergence of MERS

The first MERS case was reported in Saudi Arabia in September 2012. From September 2012 to July 2015, 1,368 laboratory-confirmed cases have been reported to the World Health Organization (WHO), at least 487 of which resulted in death. A total of 26 countries in the Middle East, Africa, Europe, Asia, and North America have had at least one MERS case. Approximately 75% of cases, however, have been from Saudi Arabia. All cases have been directly or indirectly linked to the Middle East. The United States has only had two cases, both in travelers.[169] This information can be seen in Figure 5.4 below from the WHO.

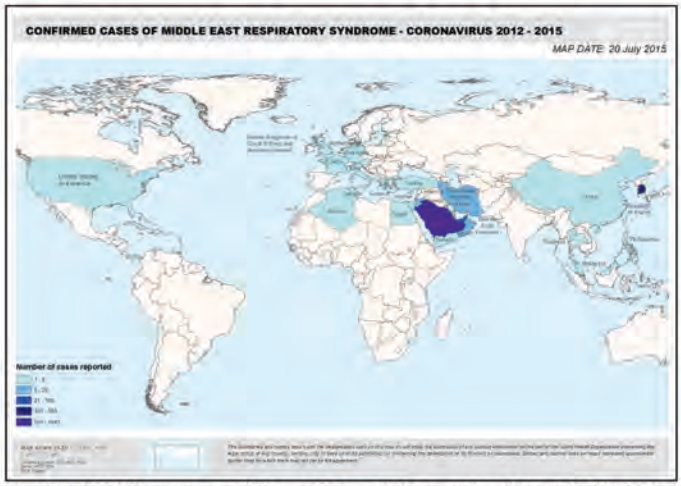

Figure 5.4. Confirmed MERS cases around the world since 2012, reproduced from The World Health Organization, Summary of Current Situation.[170]

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168 The Centers for Disease Control and Prevention. Middle East Respiratory Syndrome (MERS). http://www.cdc.gov/coronavirus/mers/. Last Update June 22, 2015. Accessed July 2015.

169 The World Health Organization (2015b) Summary of Current Situation, Literature Update and Risk Assessment. *Middle East respiratory syndrome coronavirus (MERS-CoV)* 15: 1-7

170 The World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) maps and epicurves. http://www.who.int/csr/disease/coronavirus_infections/maps-epicurves/en/. Last Update July 2015. Accessed July 2015.

5.3.4 Morbidity and Mortality

The overall MERS case fatality rate is almost 40%.[171] The WHO reported the median age of SARS cases to be 50 years old, with a range from nine months to 99 years.[172] One study of MERS patients in Saudi Arabia found a clear relation between case fatality rates and increasing age. The data supporting this correlation is provided in Table 5.6 below.[173]

Table 5.6. Case Fatality Rates with Increasing Age[174]

Age	Case fatality rate
< 19 years	0%
< 50 years	39%
< 60 years	48%
> 60 years	75%

Studies have also found that a large proportion of MERS cases are in patients with underlying medical conditions, such as diabetes and hypertension as well as chronic renal, lung, and cardiac disease (Table 5.7).[175]

Table 5.7. Percent of MERS Patients with Underlying Medical Conditions

Source	Percent with Comorbidity
Penttinen et al. 2013[176]	73%
Assiri et al. 2013[177]	96%
Arabi et al. 2014[178]	100%

Although the disease may have existed for some time before detection, MERS is a relatively new communicable disease. Research on long-term morbidity, mortality, and economic burden of the illness is in progress as the epidemic itself is still ongoing. Cases have been localized mostly to the Middle East, although cases occurred in China, Thailand, the Philippines, and the Republic of Korea in the spring and summer months of 2015.

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171 Hussain HY (2014) Incidence and Mortality Rate of "Middle East Respiratory Syndrome"-Corona Virus (MERS-Cov), Threatens and Opportunities. *J Mycobac Dis* 5.

172 The World Health Organization (2015b) Summary of Current Situation, Literature Update and Risk Assessment. *Middle East respiratory syndrome coronavirus (MERS-CoV)* 15: 1-7

173 Assiri A *et al* (2013a) Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *The Lancet Infectious diseases* 13: 752-761

174 Ibid.

175 Ibid.

176 Penttinen PM *et al* (2013) Taking stock of the first 133 MERS coronavirus cases globally--Is the epidemic changing? *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 18

177 Assiri A *et al* (2013a) Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *The Lancet Infectious diseases* 13: 752-761

178 Arabi YM *et al* (2014) Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection. *Ann Intern Med* 160: 389-397

5.3.5 2015 Outbreak

As of the writing of this section (summer of 2015), the Republic of Korea is currently experiencing the largest known MERS outbreak outside of the Arabian Peninsula. In May 2015, an infected traveler returned from the Middle East with MERS. His travel history went unreported for over a week, openly exposing many people to the virus. [179] Since then, there have been 186 confirmed cases of MERS—185 in the Republic of Korea and one in China—of which the median age is 55 years old.[180] There have been 36 deaths reported; 91.7% of the deaths were in the elderly or patients with co-morbidities. Approximately 17,000 people were quarantined.[181]

The Republic of Korea's cultural traditions are said to have influenced the rapid transmission of MERS within the country. Customs encourage friends and family to not only visit ill patients, but provide extensive bedside care. Further, in Korea, patients tend to visit several medical facilities before admitting themselves. These dispositions increase exposure and transmission of the virus.[182] It is not surprising therefore that all of the cases in Korea have been associated with health care facilities; 14% were in medical professionals. Every patient has been connected to the index case and no cases have occurred in the general population.[183] The last MERS case was reported to the WHO on July 4, 2015 and officials believe the outbreak to under control in China and the Republic of Korea. Cases continue to be reported, however, in the Arabian Peninsula.[184]

5.4 Influenza

5.4.1 Summary

- Influenza viruses cause human outbreaks seasonally, in pandemics and via zoonotic infection from birds,
- Morbidity and mortality from influenza is difficult to measure because death is due to secondary causes,
- Five to ten percent of the population worldwide gets influenza every year, and it is associated with 250,000-500,000 deaths annually, the burden of which falls mostly on the elderly,
- Seasonal influenza causes approximately $100Bn in direct and indirect economic losses in the US annually,
- Pandemic strains have caused a handful of outbreaks in the last 100 years, and are sometimes associated with significantly more illness or deaths than seasonal strains,

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179 The World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV). http://www.who.int/emergencies/mers-cov/en/. Last Update 2015. Accessed July 2015.

180 World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) – Republic of Korea. http://www.who.int/csr/don/17-july-2015-mers-korea/en/. Last Update July 17, 2015. Accessed July 2015.

181 World Health Organization (2015) Briefing for Foreign Correspondents *MERS Outbreak*.

182 Ibid..

183 World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) – Republic of Korea. http://www.who.int/csr/don/17-july-2015-mers-korea/en/. Last Update July 17, 2015. Accessed July 2015.

184 The World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) maps and epicurves. http://www.who.int/csr/disease/coronavirus_infections/maps-epicurves/en/. Last Update July 2015. Accessed July 2015

- Pandemic strains have been noted for disproportionally harming groups other than the elderly (young adults for the 1918 pandemic and young children for the 1957 and 2009 pandemics),
- Deaths from pandemics and seasonal influenza decreased across the 20th century until the 1960s, after which time the death rate from influenza has remained relatively constant,
- Outbreaks of avian influenza have caused up to $20B in direct and indirect economic losses due to destruction of poultry flocks and lost trade,
- Avian influenza outbreaks have an unpredictable effect on human health, the worst are associated with up to 1,000 cases and 500 deaths, some cause mild illness in humans, and
- Vaccines and antivirals demonstrate significant efficacy in clinical trials, however their overall public health benefit is more difficult to measure.

5.4.2 Background

Influenza is a highly contagious viral infection of the respiratory tract caused by three orthomyxoviruses of different antigenic types —influenza A, B, and C. These influenza viruses can cause seasonal and pandemic outbreaks. According to the CDC, an influenza pandemic "can occur when a non-human (novel) influenza virus gains the ability for efficient and sustained human-to-human transmission and then spreads globally." Seasonal outbreaks, however, occur annually on predictable seasonal patterns and are caused by recirculating influenza viruses with residual immunity among the population. Both seasonal and pandemic outbreaks spread through human-to-human transmission. Sporadically, avian influenza viruses, which do not normally infect or transmit through humans, will cross species barriers and cause an outbreak in the human population.[185]

Influenza type A can infect a variety of animal hosts and is further divided into subgroups based on its surface proteins (e.g., H1N1, H3N2, H5N1). This genetic variation allows type A viruses to cause pandemic outbreaks, dominate seasonal epidemics, and cross species barriers.[186] Type B viruses have a more limited host range and limited variation, and therefore, do not cause pandemic outbreaks. Virus type C causes only mild symptoms in humans and does not contribute to epidemics.[187] Influenza virus is consistently one of the leading causes of illness in the United States and is associated with significant mortality; it is among the US Centers for Disease Control and Prevention's (CDC) top priorities.[188]

5.4.3 Seasonal Influenza

Seasonal influenza viruses circulate through the population causing annual epidemics during the winter months in temperate climates, such as the United States, and unpredictable epidemics in tropical regions. The World Health Organization (WHO) estimates that 5%-10% of adults and 20%-30% of children

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185 Centers for Disease Control and Prevention. Influenza (Flu). http://www.cdc.gov/flu/. Last Update 2015. Accessed May 2015.

186 Centers for Disease Control and Prevention. Types of Influenza Viruses. http://www.cdc.gov/flu/about/viruses/types.htm. Last Update 2014. Accessed May 2015.

187 Ibid.

188 Centers for Disease Control and Prevention. Influenza (Flu). http://www.cdc.gov/flu/. Last Update 2015. Accessed May 2015.

worldwide are infected with influenza each year. Of those illnesses, three to five million develop into severe cases, which result in 250,000 to 500,000 deaths annually.[189]

5.4.4 Morbidity and Mortality of Seasonal Influenza

In developed countries, approximately 1.2 in 10,000 persons die annually as a result of influenza.[190] Industrialized countries with established surveillance systems provide the majority of case data so these attack rates could be substantially underestimated for developing, tropical countries with limited financial and technical resources.[191] Globally, influenza drives the loss of 19.2 million disability-adjusted life years (DALYs) annually (16.9 million-21.5 million), as estimated in the Global Burden of Disease Study in 2010. This statistic is equivalent to 279 DALYs (245-311 DALYs) per 100,000 people worldwide.[192]

According to the National Strategy for Pandemic Influenza by the Homeland Security Council, an average of over 200,000 hospitalizations and 36,000 deaths are caused each year by seasonal influenza in the US alone.[193] Anywhere from 5-20% of the population, or 15 to 60 million Americans, is infected with the influenza virus annually.[194] Death tolls range from 1.4 to 16.7 deaths per 100,000 persons, exceeding a total of 49,000 lives.[195] A CDC study estimated that, since the 1980s, the hospitalization rate has ranged from 150,000 to 431,000 people per year.[196] Seasonal influenza is consistently ranked in the top ten overall leading causes of death in the United States.[197] After convalescence, however, no long term morbidities are associated with influenza.[198]

5.4.5 Uncertainty When Determining Influenza-Associated Morbidity and Mortality

The CDC is uncertain on exactly how many people become infected with or die from influenza each year. States are required to report influenza-related deaths in children under 18 years old only, leaving all other cases and deaths untracked. Seasonal influenza is rarely listed as a cause of death on adults' death certificates. Additionally, death often occurs weeks after initial infection when patients are no longer symptomatic and the virus cannot be detected. Some diagnostic tests can even produce false negative results due to their low sensitivity.[199] For these reasons, the CDC must estimate the number of annual cases and deaths caused by influenza. The estimate is typically based on deaths related to pneumonia and influenza (P&I) to account for the fact that although influenza is never confirmed as the cause of death, pneumonia is most often the underlying cause.[200] Excess P&I deaths, however, only account for approximately 25% of actual influenza-related mortality.

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189 World Health Organization. Influenza (Seasonal). http://www.who.int/mediacentre/factsheets/fs211/en/. Last Update March 2014. Accessed May 2015.

190 Lagace-Wiens PR *et al* (2010) Influenza epidemiology--past, present, and future. *Crit Care Med* 38: 1-9

191 World Health Organization (2005) Influenza Vaccines. *WHO Position Paper* 33: 279-287

192 Institute for Health Metrics and Evaluation (2013) The Global Burden of Disease: Generating Evidence, Guiding Policy. 1-50

193 Gerberding JL, Centers for Disease Control and Prevention. (2005) Avain Influenza: Preparing for a possible Influenza Pandemic

194 Fiore AE *et al* (2010) *Prevention and control of influenza with vaccines : recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010*. Atlanta, Ga. : Dept. of Health and Human Services, Centers for Disease Control and Prevention.

195 Centers for Disease C, Prevention (2010) Estimates of deaths associated with seasonal influenza --- United States, 1976-2007. *MMWR Morbidity and mortality weekly report* 59: 1057-1062

196 Thompson WW *et al* (2004) Influenza-associated hospitalizations in the United States. *Jama* 292: 1333-1340

197 Centers for Disease Control and Prevention. (2015c) Leading Causes of Death.

198 Rothberg MB, Haessler SD (2010) Complications of seasonal and pandemic influenza. *Crit Care Med* 38: e91-97

199 Centers for Disease Control and Prevention. Estimating Seasonal Influenza-Associated Deaths in the United States: CDC Study Confirms Variability of Flu. http://www.cdc.gov/flu/about/disease/us_flu-related_deaths.htm. Last Update March 2015. Accessed May 2015.

200 World Health Organization (2005) Influenza Vaccines. *WHO Position Paper* 33: 279-287

One estimation method for influenza deaths is excess mortality, which is the difference between mortality rates during an influenza epidemic and the standard baseline rate in the absence of an influenza outbreak. While there is never a total absence of influenza illness, comparing the rate of illness during a predictable seasonal outbreak to the baseline rate during the summer months when there is no outbreak provides an estimation for the illnesses being caused by the outbreak. For example, during the 2013– 2014 influenza season the percentage of deaths attributed to P&I peaked at 8.7%. The baseline rate of P&I related deaths that occur during a non-outbreak time period, however, was about 7% and hence, the difference of 1.7% of P&I related deaths can be attributed to the seasonal influenza outbreak.[201]

The excess index can also be applied to the number of outpatient visits to evaluate excess morbidity.[202] The national baseline for the percentage of outpatient visits is the mean percentage of P&I visits during non-outbreak weeks for the previous three seasons plus two standard deviations and is usually about 2.0%. The baseline for mortality is calculated using a periodic regression model applied to data from the previous five years. It ranges from 6-8%, but is usually around 7.5% during seasonal influenza months.[203] Excess morbidity and mortality are not intended to provide exact statistics, but serve as an indicator of the season's relative severity. Hospitalization rates are not reported as an excess index, but instead as laboratory-confirmed statistics.

5.4.6 Consequences in Special Populations

Influenza harms young children and the elderly more than older children and non-elderly adults. The WHO reports that excess mortality attributed to influenza ranges from three to 15 per 10,000 Americans older than 65 years. In the general population, excess morbidity is approximately 1.2 in 10,000 persons per year.[204] Excess hospitalization averages at 100 per 10,000 for children under six months old, but only four per 10,000 children once they are more than five years old.[205] Persons in the age group from five to 49 years have the lowest hospitalization rate.[206]

Seasonal influenza disproportionally kills the elderly, who suffer approximately 80-90% of the mortality observed.[207] This trend is evident in Figure 5.5, from a CDC sponsored study on the epidemiology of seasonal influenza, giving both hospitalization and mortality rates.

Influenza cases typically resolve within two weeks, however severe cases or cases in elderly or at-risk populations may lead to additional complications. Complications include pneumonia, bronchitis, and exacerbation of existing pulmonary and respiratory diseases and could lead to death. Resolved cases, however, are not associated with long-term morbidity.[208]

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201 Centers for Disease Control and Prevention. (2014b) Influenza Activity — United States, 2013–14 Season and Composition of the 2014–15 Influenza Vaccines. *Morbidity and Mortality Weekly Report*, Vol. 63, pp. 483-490.

202 Simonsen L (1999) The global impact of influenza on morbidity and mortality. *Vaccine* 17 Suppl 1: 3-10

203 Centers for Disease Control and Prevention. Overview of Influenza Surveillance in the United States. Last Update Accessed June 2015.

204 Lagace-Wiens PR *et al* (2010) Influenza epidemiology--past, present, and future. *Crit Care Med* 38: 1-9

205 World Health Organization (2005) Influenza Vaccines. *WHO Position Paper* 33: 279-287

206 Thompson WW *et al* (2004) Influenza-associated hospitalizations in the United States. *Jama* 292: 1333-1340

207 Dawood FS *et al* (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infectious Diseases* 12: 687-695

208 Rothberg MB, Haessler SD (2010) Complications of seasonal and pandemic influenza. *Crit Care Med* 38: e91-97

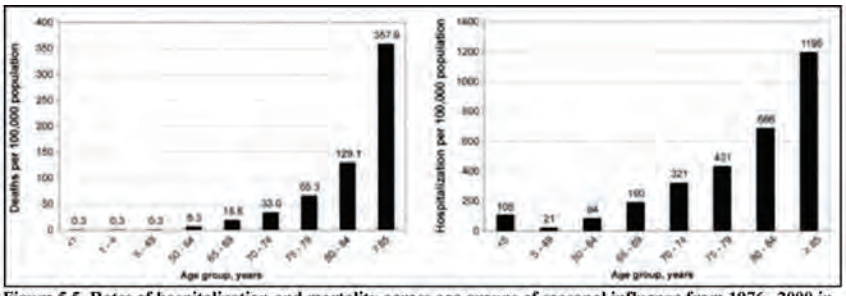

Figure 5.5. Rates of hospitalization and mortality across age groups of seasonal influenza from 1976–2000 in the US, reproduced from Thompson et. al.[209]

Table 5.8 and Table 5.9 below present several studies' estimated annual mortality rate and hospitalization rate, respectively, of seasonal influenza.

Table 5.8. Studies on the Influenza-Associated Mortality Rate (Per 100,000 Persons) Across Age Groups of Seasonal Influenza Annually in the US

Source	All ages	< 1 year	< 5 years	< 65 years	≥ 65 years
Thompson et al. 2003[210]	3.1	0.3	0.2	-	22.1
Simonsen et al. 2000[211]	2.5	-	-	0.49	18.7
CDC 2010[212]	2.4	-	-	0.4	17.0

Table 5.9. Studies on the Influenza-Associated Hospitalization Rate (Per 100,000 Persons) Across Age Groups of Seasonal Influenza Annually in the US

Source	All ages	< 1 year	> 5 years	< 65 years	≥ 65 years
Thompson et al. 2004[213]	37	-	26.3	13	205
Simonsen et al. 2000[214]	49	-	-	33	174
Zhou et al. 2012[215]	-	151	94	-	309

209 Thompson WW *et al* (2006) Epidemiology of seasonal influenza: use of surveillance data and statistical models to estimate the burden of disease. *J Infect Dis* 194 Suppl 2: S82-91

210 Thompson WW *et al* (2003) Mortality associated with influenza and respiratory syncytial virus in the United States. *Jama* 289: 179-186

211 Simonsen L *et al* (2000) The impact of influenza epidemics on hospitalizations. *J Infect Dis* 181: 831-837

212 Centers for Disease C, Prevention (2010) Estimates of deaths associated with seasonal influenza --- United States, 1976-2007. *MMWR Morbidity and mortality weekly report* 59: 1057-1062

213 Thompson WW *et al* (2004) Influenza-associated hospitalizations in the United States. *Jama* 292: 1333-1340

214 Simonsen L *et al* (2000) The impact of influenza epidemics on hospitalizations. *J Infect Dis* 181: 831-837

215 Zhou H *et al* (2012) Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993-2008. *Clin Infect Dis* 54: 1427-1436

5.4.7 Influence of Influenza Virus Type

Since 1968, influenza A, both H1N1 and H3N2, and influenza B have all co-circulated to cause seasonal epidemics. Seasons dominated by influenza A H3N2 tend to cause greater excess mortality than influenza A H1N1 and B seasons.[216] From 1990-1999, overall influenza-related mortality ranged from 17,000 to 51,000 deaths in a season. Around 90% of seasons were predominated by H3N2 strains and thus, influenza-associated mortality rose during that time compared to an average year. The increase, however, can also be partly associated with the large proportion of the population that was over 65 years old and tend to suffer more from influenza infections.[217]

5.4.8 Recent Influenza Seasons

In the past decade, both seasonal and pandemic strains have caused fluctuating outbreaks, as can be seen in Figure 5.6 and Figure 5.7 below. From 2007– 2008, the H3N2 seasonal virus predominated in the United States. That season saw the greatest mortality and hospitalization rates of the previous four years, at 83 deaths of children under four years old. The percentage of the population with P&I related deaths peaked at 9.1%, which is well above the normal baseline of 7.6%.[218]

The following influenza season, from 2008– 2009, was less severe and can be attributed to the H1N1 dominant strain that typically causes more mild outbreaks. There were only 45 pediatric deaths and a hospitalization rate of 2.8 per 10,000 children. Outpatient visits peaked at 3.7%, well over the baseline of 2%, and the weekly percentage of deaths attributed to P&I peaked at 7.6%, which is at the baseline of 7.6%.[219]

A pandemic virus emerged the following year. The 2009 H1N1 pandemic strain was unusually severe, causing an extended outbreak from April 2009 through May 2010. Infections persisted through the summer months, speaking to the strain's augmented pathogenicity and transmissibility. Excess mortality peaked at 8.1% in November and again at 8.2% in January, exceeding the national baseline for thirteen weeks straight. The pandemic resulted in the greatest number of patient visits of any year since influenza surveillance began in 1997.[220] Seasonal influenza typically burdens the elderly population the most with 80-90% of the mortality. This pandemic, however, affected children and young adults much more substantially. An estimated 80% of deaths were in people under 65 years old.[221] There were 344 reported pediatric deaths.[222]

By the 2010– 2011 season, the pandemic H1N1 strain continued to circulate. The H3N2 strain and influenza B were also widely distributed, resulting in a more balanced attack rate among all age groups. The season was significantly less severe than the previous pandemic year, but still worse than the

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216 Simonsen L (1999) The global impact of influenza on morbidity and mortality. *Vaccine* 17 Suppl 1: 3-10

217 Fiore AE *et al.* (2007) Prevention and Control of Influenza : Recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity and Mortality Weekly Reports Recommendations and Reports.* Centers for Disease Control and Prevention, Atlanta, GA, Vol. 56, pp. 1-54.

218 Centers for Disease Control and Prevention. (2008) Influenza Activity --- United States and Worldwide, 2007--08 Season. *Morbidity and Mortality Weekly Report*, Vol. 57, pp. 692-697.

219 Centers for Disease Control and Prevention. (2009) Update: Influenza Activity-- United States, September 28, 2008- April 4, 2009, and Composition of the 2009-2010 Influenza Vaccine *Morbidity and Mortality Weekly Report*, Vol. 58, pp. 369-374.

220 Centers for Disease Control and Prevention. (2010) Update: Influenza Activity United States, 2009--10 Season. *Morbidity and Mortality Weekly Report*, Vol. 59, pp. 901-908.

221 Dawood FS *et al* (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infectious Diseases* 12: 687-695

222 Centers for Disease Control and Prevention. (2010) Update: Influenza Activity United States, 2009--10 Season. *Morbidity and Mortality Weekly Report*, Vol. 59, pp. 901-908.

previous 2008– 2009 endemic. There were 105 confirmed pediatric deaths. Outpatient visits peaked at 4.6%, the hospitalization rate was 4.38 per 10,000 children, and excess mortality surpassed the epidemic threshold for 13 consecutive weeks, peaking at 8.9%.[223]

Once again in the 2011– 2012 season, all three strains—H1N1, H3N2, and type B— circulated, but H3N2 was the predominant virus. This mild season was noted for lower hospitalization rates, outpatient visits, and deaths than previous years. The hospitalization rate amounted to only 0.86 per 10,000 people and patient visits dropped to the lowest since point 1997 by meeting, but not exceeding the threshold baseline of 2.4%. The P&I associated death rate surpassed the baseline for one short week, at 7.9%, and only 26 pediatric deaths were reported.[224]

The 2012– 2013 epidemic was more severe. All three virus types circulated, but H3N2 was the main circulating virus. Patient visits soared past endemic thresholds for 15 weeks at a high of 6.1%, much greater than the national baseline of 2%, and hospitalization rates escalated to 4.43 per 10,000 people. Mortality also surpassed the baseline for 13 weeks, reaching 9.9%. There were 149 influenza-associated pediatric deaths.[225]

From 2013– 2014, the pandemic influenza A H1N1 strain co-circulated with limited H3N3 and B strains. This was the first season since 2009 that the pandemic strain predominantly recirculated, this time as a seasonal virus. Although there were fewer deaths and hospitalizations than typically observed with an H1N1 season, adults were once again at a higher-risk for influenza. Those aged 50-64 experienced 5.43 hospitalizations per 10,000 persons compared to the aggregate rate of 3.56 per 10,000 across all age groups. Outpatient visits exceeded the baseline for 15 consecutive weeks, peaking at 4.6%, and excess mortality exceeded for eight weeks, maxing out at 8.7%. There were 96 pediatric deaths reported.[226]

Table 5.10 below compiles key CDC statistics from the Emerging Infections Program (EIP) for recent influenza seasons in the United States. Beginning in 2009, EIP was expanded to surveille 26 million more Americans, 8.5% of the population.[227] This new FluSurv-NET program may account for some of the differences in comparable statistics.

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[223] Centers for Disease Control and Prevention. (2011) Update: Influenza Activity -- United States, 2010--11 Season, and Composition of the 2011--12 Influenza Vaccine. *Morbidity and Mortality Weekly Report*, Vol. 60, pp. 705-712.

[224] Centers for Disease Control and Prevention. (2012) Update: Influenza Activity — United States, 2011–12 Season and Composition of the 2012–13 Influenza Vaccine. *Morbidity and Mortality Weekly Report*, Vol. 61, pp. 414-420.

[225] Centers for Disease Control and Prevention. (2013b) Influenza Activity — United States, 2012–13 Season and Composition of the 2013–14 Influenza Vaccine. *Morbidity and Mortality Weekly Report*, Vol. 62, pp. 473-479.

[226] Centers for Disease Control and Prevention. (2014b) Influenza Activity — United States, 2013–14 Season and Composition of the 2014–15 Influenza Vaccines. *Morbidity and Mortality Weekly Report*, Vol. 63, pp. 483-490.

[227] Centers for Disease Control and Prevention. (2011) Update: Influenza Activity -- United States, 2010--11 Season, and Composition of the 2011--12 Influenza Vaccine. *Morbidity and Mortality Weekly Report*, Vol. 60, pp. 705-712.

Table 5.10 Influenza-Associated Statistics as Reported to the CDC Annually for Past Influenza Seasons in the United States

Season	Dominant Strain	Outpatient Visits	P&I Related Mortality	Hospitalization Rate ≤ 4 years	Hospitalization Rate ≥ 65 years	Pediatric Deaths
2007-2008[228]	H3N2	6.0%	9.1%	40.3	-	83
2008-2009[229]	H1N1	3.7%	7.6%	28.0	10.0	45
2009-2010[230]	pH1N1	7.6%	8.2%	83.0	32.0	344
2010-2011[231]	H3N2/ H1N1	4.6%	8.9%	43.8	62.5	105
2011-2012[232]	H3N2	2.4%	7.9%	14.2	30.4	26
2012-2013[233]	H3N1	6.1%	9.9%	66.2	191.2	149
2013-2014[234]	H1N1	4.6%	8.7%	46.9	88.1	96

Hospitalization rate is given per 100,000 persons.

Figure 5.6 below from the CDC presents the percentage of influenza-associated outpatient visits in the US by surveillance week over several past influenza seasons. Figure 5.7 depicts P&I attributable deaths in influenza seasons since 2009. Both figures plot statistics in regards to their national baselines so that excess morbidity and mortality can be visualized. The fluctuation between yearly seasons and pandemic outbreaks are apparent.

228 Centers for Disease Control and Prevention. (2008) Influenza Activity --- United States and Worldwide, 2007--08 Season. *Morbidity and Mortality Weekly Report*, Vol. 57, pp. 692-697.

229 Centers for Disease Control and Prevention. (2009) Update: Influenza Activity-- United States, September 28, 2008- April 4, 2009, and Composition of the 2009-2010 Influenza Vaccine *Morbidity and Mortality Weekly Report*, Vol. 58, pp. 369-374.

230 Centers for Disease Control and Prevention. (2010) Update: Influenza Activity United States, 2009--10 Season. *Morbidity and Mortality Weekly Report*, Vol. 59, pp. 901-908.

231 Centers for Disease Control and Prevention. (2011) Update: Influenza Activity --- United States, 2010--11 Season, and Composition of the 2011--12 Influenza Vaccine. *Morbidity and Mortality Weekly Report*, Vol. 60, pp. 705-712.

232 Centers for Disease Control and Prevention. (2012) Update: Influenza Activity — United States, 2011–12 Season and Composition of the 2012–13 Influenza Vaccine. *Morbidity and Mortality Weekly Report*, Vol. 61, pp. 414-420.

233 Centers for Disease Control and Prevention. (2013b) Influenza Activity — United States, 2012–13 Season and Composition of the 2013–14 Influenza Vaccine. *Morbidity and Mortality Weekly Report*, Vol. 62, pp. 473-479.

234 Centers for Disease Control and Prevention. (2014b) Influenza Activity — United States, 2013–14 Season and Composition of the 2014–15 Influenza Vaccines. *Morbidity and Mortality Weekly Report*, Vol. 63, pp. 483-490.

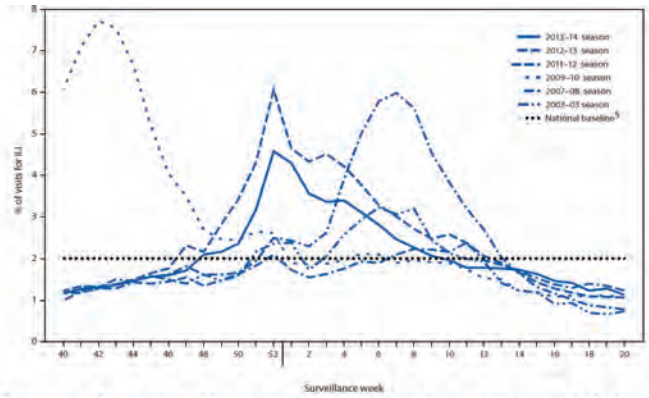

Figure 5.6. Percentage of outpatient visits attributable to influenza by surveillance week during past influenza seasons in the United States, as reported to the CDC, reproduced from Centers from Disease Control and Prevention, Influenza Activity — United States, 2013–14 Season and Composition of the 2014–15 Influenza Vaccines.[235]

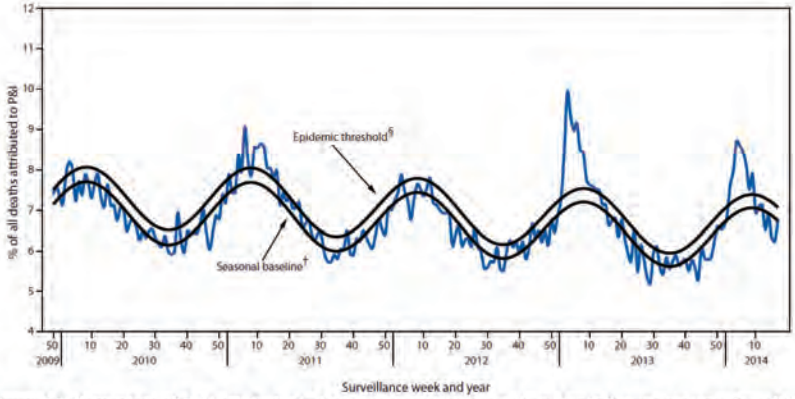

Figure 5.7. Percentage of P&I attributable deaths by surveillance week during influenza seasons since 2009 in the United States. Figure reproduced from Centers from Disease Control and Prevention, Influenza Activity — United States, 2013–14 Season and Composition of the 2014–15 Influenza Vaccines.[236]

5.4.9 Economic Burden of Seasonal Influenza

The US public health infrastructure is burdened with 24.7 million (19.9-30.1 million) cases of seasonal influenza annually. These cases generate approximately 31.4 million (22.6-43.5 million) outpatient visits

[235] Ibid.
[236] Ibid.

and 3.1 million days of hospitalization, producing approximately a $10.4 billion ($4.1- $22.2 billion) burden of direct medical costs from annual influenza endemics, according to a 2003 study by the Immunization Service Division and Division of Viral Diseases at the CDC.[237] Additionally, anywhere from $8.7 to $31 billion in earnings is lost annually due to decreases in productivity and loss of life from the estimated 610,000 (360,000- 953,000) DALYs lost. In total, the annual costs of seasonal influenza amount to $87.1 billion ($47.2- $149.5 billion) in direct and indirect costs in the United States.[238] Table 5.11 below displays several studies' estimated economic harm of seasonal influenza on the US. Echoing these data, the WHO reported that France and Germany may spend anywhere from $1 million to $6 million per 100,000 people annually on influenza outbreaks.[239]

Table 5.11. Studies on the Direct Medical Cost and Total Economic Burden of Seasonal Influenza Annually on the US

Source	Medical Costs (per year)	Economic Burden (per year)
Office of Technology Assessment 1981[240]	$1- $3 billion	-
Molinari et al. 2007[241]	$10.4 billion	$87.1 billion
Mao et al. 2012[242]	$10.3 billion	$29.1 billion

5.4.10 Pandemic Influenza

In addition to the annual burden of seasonal influenza, several pandemic strains have caused additional morbidity and mortality over the past century. Over the past 300 years, ten pandemics are known to have occurred, three of which were in the 20th century.[243]

The 1918 influenza pandemic, also known as the "Spanish Flu" (the exact location of origin was never determined), was the deadliest outbreak in modern history.[244] The H1N1 outbreak occurred in three waves beginning in March 1918. The second and most severe wave occurred concurrently in North America, Africa, and Europe in August 1918.[245] Over the next six months, anywhere from 20-40% of the global population was infected with influenza and approximately 500 million people became ill.[246] Estimates on the total mortality ranges from 20 to 100 million worldwide, but most estimates suggest approximately 50

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237 Molinari NA *et al* (2007) The annual impact of seasonal influenza in the US: measuring disease burden and costs. *Vaccine* 25: 5086-5096s

238 Ibid.

239 World Health Organization (2005) Influenza Vaccines. *WHO Position Paper* 33: 279-287

240 US Congress: Office of Technology Assessment: Cost-effectiveness of Influenza Vaccination. Washington, DC: GPO; 1981

241 Molinari NA *et al* (2007) The annual impact of seasonal influenza in the US: measuring disease burden and costs. *Vaccine* 25: 5086-5096

242 Mao L *et al* (2012) Annual economic impacts of seasonal influenza on US counties: spatial heterogeneity and patterns. *Int J Health Geogr* 11: 16

243 Osterholm MT (2005) Preparing for the next pandemic. *N Engl J Med* 352: 1839-1842

244 Lagace-Wiens PR *et al* (2010) Influenza epidemiology--past, present, and future. *Crit Care Med* 38: 1-9

245 Ibid.

246 National Institute of Allergy and Infectious Diseases. (2011) Pandemic Flu History. Department of Health & Human Services, Washington, DC.

million deaths.[247, 248] In the United States, between 550,000 and 675,000 deaths were attributed to the pandemic.[249, 250]

Unlike seasonal influenza, the highest rates of morbidity and mortality were observed in young, healthy adults instead of the elderly.[251] The case fatality rate was greater than 2.5%, compared to later pandemics that were less than 0.1%.[252] The reason for the severity of the outbreak is unclear, considering the attack rate and age distribution was similar to other pandemics. Moreover, H1N1 outbreaks are typically associated with lower levels of morbidity and mortality.[253] In total, anywhere from 1% to 3% of the global population died as a result of this pandemic.[254]

In February 1957 another pandemic strain emerged, influenza A H2N2 originating from China. The virus spread rapidly around the world and to the United States, with most related excess mortality occurring between September 1957 and March 1958.[255] Morbidity in children exceeded 50%.[256] Approximately 70,000 deaths occurred in the United States and two million worldwide, which amounts to approximately 0.07% of the population worldwide dying from influenza-associated causes.[257]

Just over a decade later, a new influenza A H3N2 strain emerged in Hong Kong in July 1968. The virus was slow moving, it didn't reach the United States until December and Europe the following year.[258] Attack rates peaked at 40% in children, but mortality rates were highest in the elderly. The excess mortality amounted to approximately 33,800 deaths in the United States, mild for a pandemic outbreak.[259] The 1968-1969 outbreak had the lowest mortality rate of any other pandemic of the century, possibly due to partial immunity from exposure to the 1957 pandemic strain and improved medical treatment.[260] Overall, approximately 0.03% of the population worldwide died from influenza-associated causes during the 1968 pandemic.[261]

Table 5.12 below presents the age distribution of mortality attributable to influenza during 20th century influenza pandemics. Figure 5.8 shows antigenic type and associated age-based mortality of pandemics through 1995. Interpandemic seasons are also included for comparison.

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247 Taubenberger JK, Morens DM (2006) 1918 Influenza: the mother of all pandemics. *Emerging infectious diseases* 12: 15-22
248 Noymer A, Garenne M (2000) The 1918 influenza epidemic's effects on sex differentials in mortality in the United States. *Population and development review* 26: 565-581
249 National Institute of Allergy and Infectious Diseases. (2011) Pandemic Flu History. Department of Health & Human Services, Washington, DC.
250 Crosby, A. 1989. America's Forgotten Pandemic: The Influenza of 1918. Cambridge: Cambridge University Press.
251 Lagace-Wiens PR *et al* (2010) Influenza epidemiology--past, present, and future. *Crit Care Med* 38: 1-9
252 Taubenberger JK, Morens DM (2006) 1918 Influenza: the mother of all pandemics. *Emerging infectious diseases* 12: 15-22
253 Simonsen L (1999) The global impact of influenza on morbidity and mortality. *Vaccine* 17 Suppl 1: 3-10
254 Murray CJ *et al* (2006) Estimation of potential global pandemic influenza mortality on the basis of vital registry data from the 1918-20 pandemic: a quantitative analysis. *Lancet* 368: 2211-2218
255 National Institute of Allergy and Infectious Diseases. (2011) Pandemic Flu History. Department of Health & Human Services, Washington, DC.
256 Cox NJ, Subbarao K (2000) Global epidemiology of influenza: past and present. *Annual review of medicine* 51: 407-421
257 Mathews JD *et al* (2009) Understanding influenza transmission, immunity and pandemic threats. *Influenza and other respiratory viruses* 3: 143-149
258 Lagace-Wiens PR *et al* (2010) Influenza epidemiology--past, present, and future. *Crit Care Med* 38: 1-9
259 Cox NJ, Subbarao K (2000) Global epidemiology of influenza: past and present. *Annual review of medicine* 51: 407-421
260 Lagace-Wiens PR *et al* (2010) Influenza epidemiology--past, present, and future. *Crit Care Med* 38: 1-9
261 Mathews JD *et al* (2009) Understanding influenza transmission, immunity and pandemic threats. *Influenza and other respiratory viruses* 3: 143-149

Table 5.12. Influenza-Associated Mortality (per 100,000) Across Age Groups During Pandemics in the US [262]

Season	Pandemic Strain	All ages	< 65 years	≥ 65 years
1918-1919	H1N1	529	546	166
1957-1958	H2N2	39	15	273
1968-1969	H3N2	8.1	4.3	44

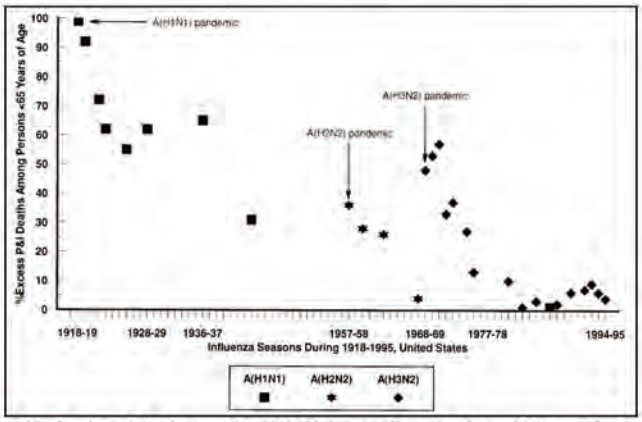

Figure 5.8. Age distribution of deaths associated with influenza A pandemics and interpandemic seasons in the United States, 1918-1995, reproduced from Simonsen et. al[263]

In 2009, a pandemic H1N1 strain quickly circulated through 74 countries during the summer months, an unusual time for the virus.[264] The excess mortality is estimated between 152,000 and 575,000 people worldwide. Populations in Africa and Asia suffered approximately 50% of the deaths, possibly due to the limited availability of medical treatment, presence of underlying health conditions, lower qualities of care, and fragile nutritional status.[265] In the initial stages of the pandemic, Mexico was spending an estimated $57 million per day trying to control and treat the virus. The World Bank claimed a total $3 trillion loss from the burden of the pandemic.[266]

In the United States, from 43 to 89 million people became infected with pandemic H1N1, resulting in 9,000 to 18,300 deaths. Point of care testing used to identify the virus is less sensitive on pandemic strains

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262 Simonsen L *et al* (1998) Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. *J Infect Dis* 178: 53-60

263 Ibid.

264 National Institute of Allergy and Infectious Diseases. (2011) Pandemic Flu History. Department of Health & Human Services, Washington, DC.

265 Dawood FS *et al* (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infectious Diseases* 12: 687-695

266 Lagace-Wiens PR *et al* (2010) Influenza epidemiology--past, present, and future. *Crit Care Med* 38: 1-9

and therefore, infection rates also could have been much greater.[267] Children experienced significantly higher mortality and hospitalization rates than the elderly, who are normally the most affected population group, as can be seen in Table 5.13. The hospitalization rate was 8.3 per 10,000 among ages zero to four years and 3.4 per 10,000 among ages 5-17 years while only 3.2 per 10,000 for those over 65 years.[268] The severity and extent of the outbreak was curtailed by the rapid vaccination of more than 80 million people. The H1N1 pandemic strain continues to circulate today as a seasonal human flu virus.

Table 5.13. Statistics on the Number of Influenza Cases, Influenza-Related Mortality, and Hospitalization Rate Resulting from the 2009 H1N1 Pandemic in the US

Season	Case Count	Death Toll	Hospitalization Rate (per 10,000 persons)		
			< 4 years	5-17 years	> 65 years
2009	43-89 million	9,000-18,000	8.3	3.4	3.2

5.4.11 Pandemic Threats

Over the past century, there were also several instances of newly emerged influenza strains that were feared to cause a pandemic but did not. In 1976, a swine strain with similarities to the 1918 pandemic strain emerged at Fort Dix, New Jersey. Due to a robust vaccination campaign and other unknown factors, the virus never became widespread. Then again in May of 1977, a new virus type surfaced in China and began to rapidly spread around the world. The strain was similar to strains circulating prior to 1957. Many adults had already developed immunity to the virus, which limited outbreaks to children mainly and prevented a major pandemic. Decades later in 1997, once again in China, a novel H5N1 virus began to infect young adults directly from chickens. Over one million chickens were culled to successfully prevent further spread of the avian flu.[269]

5.4.12 Avian Influenza

Within the past two decades, avian influenza A has caused substantial morbidity and mortality in humans. Of the many existing strains, H5 and H7 subtypes have been the primary transmission sources from birds to humans. H5N1 viruses are endemic in Asia and Africa while subtype H7 circulates across Europe and North America.[270] Avian influenza A viruses are either highly pathogenic (HPAI) or have low pathogenicity (LPAI) based on the degree of infection caused by their molecular characteristics. While bird-to-human transmission of avian influenza is a persistent issue, sustained human-to-human transmission of avian strains has not been observed.[271]

The H5N1 virus first infected humans in Hong Kong in 1997 stemming from an outbreak in poultry. It reemerged in mainland China in 2003 and quickly spread through wild bird migration and domestic poultry trading among Asia, Africa, and Europe. The virus remains endemic in birds, with sporadic

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267 National Institute of Allergy and Infectious Diseases. (2011) Pandemic Flu History. Department of Health & Human Services, Washington, DC.

268 Centers for Disease Control and Prevention. (2010) Update: Influenza Activity United States, 2009--10 Season. *Morbidity and Mortality Weekly Report*, Vol. 59, pp. 901-908.

269 National Institute of Allergy and Infectious Diseases. (2011) Pandemic Flu History. Department of Health & Human Services, Washington, DC.

270 Belser JA *et al* (2009) Past, Present, and Possible Future Human Infection with Influenza Virus A Subtype H7. *Emerging Infectious Diseases* 15: 859-865

271 Peiris JS *et al* (2007) Avian influenza virus (H5N1): a threat to human health. *Clin Microbiol Rev* 20: 243-267

transmission to humans.[272] These H5N1 viruses had various genotypes of geographically-related sublineages. [273] Since reemerging in 2003, and up to July 2015, there have been 844 cases of human infection with H5N1 and 449 deaths reported to the WHO, equating to a 53% mortality rate (Figure 5.9). Sixteen countries have had outbreaks of H5N1 avian influenza; Egypt, Indonesia, and Viet Nam have experienced the most cases.[274]

H5N1 was the first avian influenza virus to continuously circulate in Asia for over 16 years.[275] According to the Food and Agriculture Organization of the United Nations, by 2008 the economic losses were estimated to have already reached $20 billion worldwide. The two US outbreaks were estimated to have cost $65 and $140 million from the loss of poultry and cost of disease control alone. The United States also committed $1.4 billion towards the international effort against H5N1.[276] The economies of countries in East and Southeast Asia suffered the most due to the affect H5N1 had on the poultry industry. From 2003 to 2005, an estimated ten billion dollars was lost from the death or culling of over 140 million birds in Southeast Asia. GDP was depleted by 0.6% to 2% among affected countries.[277]

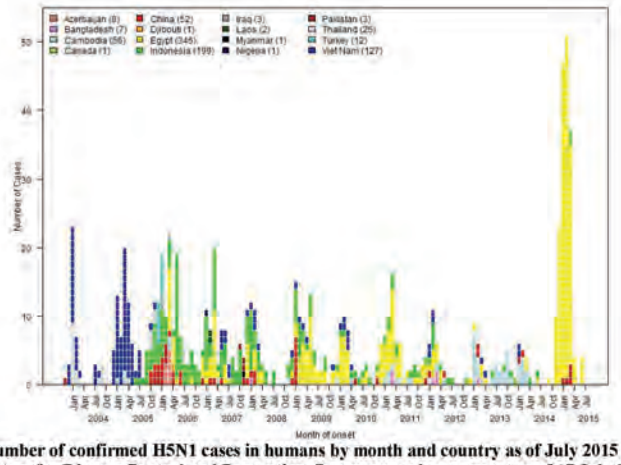

Figure 5.9. Number of confirmed H5N1 cases in humans by month and country as of July 2015 reproduced from The Centers for Disease Control and Prevention, Summary and assessment as of 17 July 2015.[278]

In March 2013, the H7N9 virus was identified as the causative agent of infections in two patients in Shanghai and one in Anhui Province, China. Spread throughout China continued through exposure to

272 The World Health Organization. Avian Influenza Fact Sheet. http://www.who.int/mediacentre/factsheets/avian_influenza/en/. Last Update March 2014. Accessed August 2015.
273 Peiris JS *et al* (2007) Avian influenza virus (H5N1): a threat to human health. *Clin Microbiol Rev* 20: 243-267
274 The World Health Organization. Cumulative number of confirmed human cases for avian influenza A(H5N1) http://www.who.int/influenza/human_animal_interface/EN_GIP_20150717cumulativeNumberH5N1cases.pdf?ua=1. Last Update July 2015. Accessed August 2015.
275 Simms L, Jeggo M (2014) Avian influenza from an ecohealth perspective. *EcoHealth* 11: 4-14
276 Commission of the European Communities (2015) *Impact Assessment Avian Influenza. 171*
277 Peiris JS *et al* (2007) Avian influenza virus (H5N1): a threat to human health. *Clin Microbiol Rev* 20: 243-267
278 The World Health Organization (2015e) Summary and assessment as of 17 July 2015. *Influenza at the human-animal interface*: 1-4

infected birds, typically in live poultry markets, but not through human-to-human contact.[279] As of July 2015, 677 cases of H7N9 with at least 275 deaths have been reported to the WHO, a 41% mortality rate (Figure 5.10). The virus remains endemic in China, but no cases have been reported outside of the mainland.[280] According to the United Nations, China experienced over $6.5 billion in losses from the H7N9 outbreak.[281]

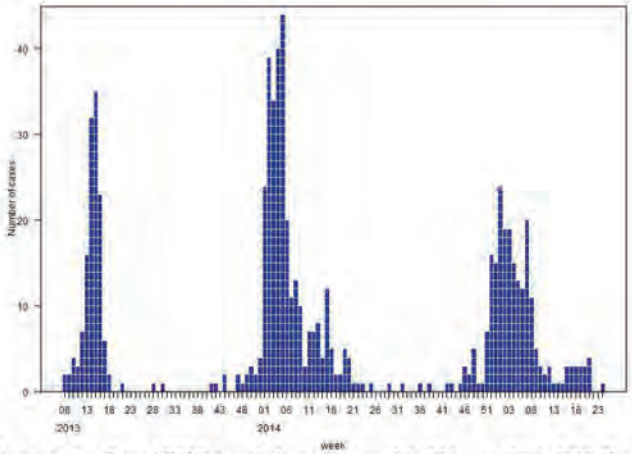

Figure 5.10. Number of confirmed H7N9 cases in humans by week in China as of July 2015. Figure reproduced from The World Health Organization, Summary and assessment as of 17 July 2015.[282]

Since 2002, other influenza H7 subtypes have caused more than 100 human infections. Subtype H7N2 caused a widespread outbreak on domestic turkey farms in the northeastern United States in 2002. One worker in Virginia became infected with the virus while birds were being culled to contain the outbreak, which proved the possibility of bird to human transmissibility in the US. A year later in New York, H7N2 was identified in an immunocompromised man who denied contact with any live poultry.[283] The transmission source remains unknown.[284] Both US cases of human H7N2 fully recovered. In the United Kingdom, several cases of H7N2 were reported in 2007; three people were hospitalized, all of whom recovered.[285]

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279 The World Health Organization. Avian Influenza Fact Sheet. http://www.who.int/mediacentre/factsheets/avian_influenza/en/. Last Update March 2014. Accessed August 2015.

280 The World Health Organization (2015e) Summary and assessment as of 17 July 2015. *Influenza at the human-animal interface*: 1-4

281 Nebehay, S. (2013) China's bird flu outbreak cost $6.5 billion. *Reuters*.

282 The World Health Organization (2015e) Summary and assessment as of 17 July 2015. *Influenza at the human-animal interface*: 1-4

283 Belser JA *et al* (2009) Past, Present, and Possible Future Human Infection with Influenza Virus A Subtype H7. *Emerging Infectious Diseases* 15: 859-865

284 Belinda Ostrowsky et al., "Low Pathogenic Avian Influenza A (H7N2) Virus Infection in Immunocompromised Adult, New York, USA, 2003," *Emerging Infectious Diseases* 18, no. 7 (July 2012): 1128–31

285 E. M. Abdelwhab, J. Veits, and T. C. Mettenleiter, "Prevalence and Control of H7 Avian Influenza Viruses in Birds and Humans," *Epidemiology and Infection* 142, no. 5 (May 2014): 896–920

Human H7N3 has been identified in Italian poultry workers in 2003, Canadian poultry workers in 2004, and one poultry worker in the United Kingdom in 2006.[286,287,288] Years later in 2012, H7N3 caused a severe outbreak in chicken farms in Mexico that resulted in two infected workers. While H7N3 is capable of human infection, few cases and no deaths have been reported; the subtype's capability within humans appears limited.[289]

The first emergence of H7N7 was in 1996 when a woman became ill after cleaning her poultry shed in England.[290] Then in 2002, a H7N7 outbreak in the Netherlands became the first avian influenza outbreak in humans since H5N1 emerged. A poultry outbreak on commercial farms led to more than 1000 people with subclinical indications, 86 human infections, and at least one death.[291] During an outbreak in Italy in 2013, three poultry workers contracted H7N7 without respiratory symptoms. Human to human transmission did not occur.[292] Transmissibility of the H7N7 strain is still not well understood.

5.4.13 Trends in Mortality from Influenza in the 20th Century

Unlike the newly emergent diseases SARS and MERS, influenza has caused human misery for centuries. Because of the lack of historical data as well as the difficulty of determining influenza mortality as previously described, little research exists on the historical trends of influenza. As discussed above (and shown in Figure 5.12), although Simonsen et al. focused on pandemic influenza, their data on mortality from intrapandemic seasons shows an overall downward trend in mortality from seasonal influenza.[293] Doshi et al. used mortality reports from *Vital Statistics of the United States* along with US Census estimates to calculate the incidence of influenza mortality over the past century during pandemic and seasonal outbreaks (Figure 5.11).[294] The *Health Sentinel* also analyzed mortality reports from *Vital Statistics of the United States* along with other statistics from the HHS to determine the incidence of influenza mortality over the past century (Figure 5.12).[295] Although both groups likely capture deaths from respiratory ailments other than influenza, both groups show a significant reduction in annual deaths from influenza. Importantly, although both papers suggest an overall drop in mortality over the past hundred years, both papers also clearly show that the downward trend has largely ceased, as mortality from influenza has been roughly the same in the last thirty years (and perhaps over the last 50). Without significant medical advances in the future, we can expect seasonal influenza to kill tens of thousands of Americans and nearly half a million people globally every year.

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286. Puzelli et al., "Serological Analysis of Serum Samples from Humans Exposed to Avian H7 Influenza Viruses in Italy between 1999 and 2003," *The Journal of Infectious Diseases* 192, no. 8 (October 15, 2005): 1318–22
287. Tweed et al., "Human Illness from Avian Influenza H7N3, British Columbia," *Emerging Infectious Diseases* 10, no. 12 (December 2004): 2196–99
288. Nguyen-Van-Tam et al., "Outbreak of Low Pathogenicity H7N3 Avian Influenza in UK, Including Associated Case of Human Conjunctivitis," *Euro Surveillance: Bulletin Européen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin* 11, no. 5 (2006): E060504.2
289. Lopez-Martinez I *et al* (2013a) Highly pathogenic avian influenza A(H7N3) virus in poultry workers, Mexico, 2012. *Emerg Infect Dis* 19: 1531-1534
290. Kurtz, et. al, "Avian Influenza Virus Isolated from a Woman with Conjunctivitis," *Lancet (London, England)* 348, no. 9031 (September 28, 1996): 901–2
291. Enserink, "Infectious Diseases. Bird Flu Infected 1000, Dutch Researchers Say," *Science (New York, N.Y.)* 306, no. 5696 (October 22, 2004): 590
292. Puzelli S *et al* (2014a) Human Infection with Highly Pathogenic A(H7N7) Avian Influenza Virus, Italy, 2013. *Emerging Infectious Diseases* 20: 1745-1749
293. Simonsen L et al (1998) Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. J Infect Dis 178: 53-60
294. Puzelli S *et al* (2014a) Human Infection with Highly Pathogenic A(H7N7) Avian Influenza Virus, Italy, 2013. *Emerging Infectious Diseases* 20: 1745-1749-945
295. Health Sentinel. The World Health Organization. Severe acute respiratory syndrome. http://www.who.int/topics/sars/en/. Last Update Accessed July 2015.

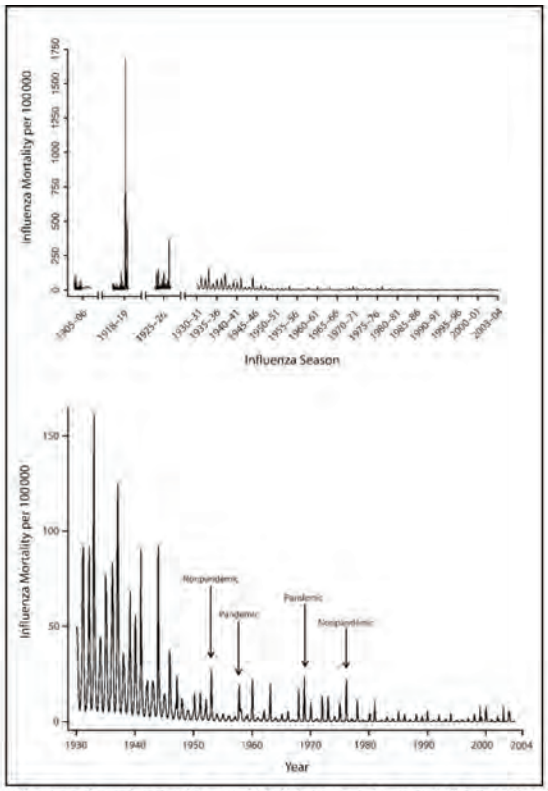

Figure 5.11. Crude influenza-classed mortality per 100,000 persons by month from 1900–2004 (top) and 1930–2004 (bottom) in the United States, as reproduced from Doshi et. al.[296]

[296] Doshi AM *et al* (2014) Trends in Recorded Influenza Mortality: United States, 1900-2004, 2009. *American Journal of Public Health* 98: 939-945

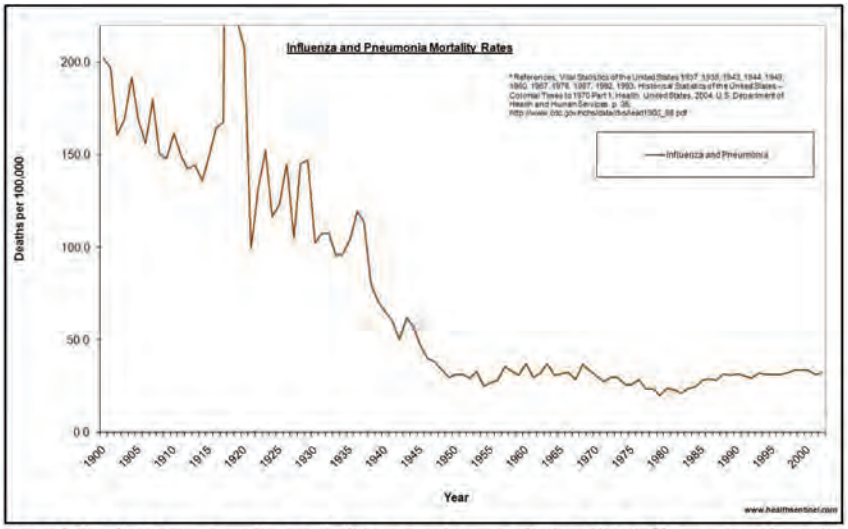

Figure 5.12. United States mortality rate for influenza and pneumonia from 1900–2002, reproduced from The *Health Sentinel*.[297]

5.4.14 Medical Countermeasures Against Influenza

Medical countermeasures (MCM) are crucial for both preparedness and response to influenza. There are two main types of influenza countermeasures: antiviral agents and vaccinations. Research on development of an influenza vaccine began soon after the virus was isolated in 1933.[298] The first wide-scale use of the vaccine occurred in 1945 during World War II among the US military. In 1960, after the 1957-1958 pandemic, the US Surgeon General recommended influenza vaccinations for high risk groups, including the elderly, pregnant women, and those with chronic conditions. Then in 2010, after the 2009 H1N1 pandemic, the Advisory Committee on Immunization Practices promoted universal influenza vaccination in persons over six months for the first time.[299]

Adamantanes and neuraminidase inhibitors are the two classes of antiviral drugs approved by the Food and Drug Administration (FDA) for use against influenza. The adamantanes, amantadine and rimantadine, are agents against influenza A and were approved for treatment in the 1966 and 1973, respectively. The Centers for Disease Control and Prevention (CDC) no longer recommends adamantanes for the treatment of seasonal influenza due to increasing resistance of circulating strains.[300] The

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297 Health Sentinel. The World Health Organization. Severe acute respiratory syndrome. http://www.who.int/topics/sars/en/. Last Update Accessed July 2015.

298 Hannoun C (2013) The evolving history of influenza viruses and influenza vaccines. *Expert review of vaccines* 12: 1085-1094

299 Osterholm MT *et al* (2012) Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *The Lancet Infectious diseases* 12: 36-44

300 Centers for Disease Control and Prevention. Use of Antivirals: Background and Guidance on the Use of Influenza Antiviral Agents. http://www.cdc.gov/flu/professionals/antivirals/antiviral-use-influenza.htm. Last Update Feb 25, 2015. Accessed October 2015.

neuraminidase inhibitors, oseltamivir and zanamivir were both approved in 1999 and function against influenza A and B.[301] Peramivir, also a neuraminidase inhibitor, was recently approved in December 2015.[302]

5.4.14.1 Efficacy

Seasonal influenza and pandemic influenza respond differently to the available MCM. This section summarizes the efficacy of the currently available MCM. Additional details and references are provided in the Supplemental Information.

5.4.14.2 Seasonal Influenza

Antivirals

There are two categories of influenza antiviral drugs, but because adamantanes are no longer recommended for treatment of seasonal influenza, they are not considered in this analysis.[303]

There are three neuraminidase inhibitors used to treat seasonal influenza: oseltamivir, zanamivir, and peramivir. Antiviral treatment is most effective when administered within 48 hours of symptom onset.[304] Antivirals can have a variety of benefits for patients suffering from seasonal influenza (see Supplemental Information on Antiviral and Vaccine Efficacy):

- Duration of symptoms is decreased by 15-40%,
- Reduction of the probability of death from influenza by five-fold, and
- Reduction in the amount of viral titer and the duration of viral shedding by 25-70%.

Antivirals can be provided as prophylaxis as well. If provided prior to exposure, antivirals prevent symptoms of seasonal influenza in 80% of patients.

Vaccines

Based on the CDC's reported adjusted overall vaccine effectiveness (the reduction in risk of needing a doctor's visit) for influenza seasons from 2005– 2015 (excluding the 2008– 2009 influenza season), the weighted average of vaccine effectiveness for seasonal influenza is 44.2%.[305] Seasonal influenza vaccination is also effective in preventing severe influenza. Each year, on average, vaccination produces, a 15% reduction in hospitalizations due to influenza illness. This result agrees with more recent studies that also show less than a 50% efficacy.[306]

One study suggests that a previous year's influenza vaccine may confer protection against current circulating viruses. During the 2012–2013 season, the vaccine effectiveness against influenza A (H3N2)

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301 Department of Health and Human Services NIoH. (2006) Development and Use of Antivirals for Pandemic Influenza *Meeting Summary* Bethesda, MD.

302 U.S. Food and Drug Administration. (2014) FDA approves Rapivab to treat flu infection. *FDA News Release*. U.S. Food and Drug Administration.

303 Centers for Disease Control and Prevention. Use of Antivirals: Background and Guidance on the Use of Influenza Antiviral Agents. http://www.cdc.gov/flu/professionals/antivirals/antiviral-use-influenza.htm. Last Update Feb 25, 2015. Accessed

304 Ibid.

305 Centers for Disease Control and Prevention. Seasonal Influenza Vaccine Effectiveness, 2005-2015. http://www.cdc.gov/flu/professionals/vaccination/effectiveness-studies.htm. Last Update June 24, 2015. Accessed Aug 11, 2015.

306 Cowling, BJ et al "Assessment of influenza vaccine effectiveness in a sentinel surveillance network 2010-13, United States." *Vaccine* 2015, article in press.

among people who received the 2012– 2013 vaccination was similar to those who received only the 2011– 2012 vaccination.[307]

5.4.14.3 Pandemic H1N1 Influenza

Antivirals

During the outbreak of pandemic H1N1 influenza, most patients were treated with oseltamivir, based on the CDC's recommendation. Of the 3,362 2009 pandemic influenza A (H1N1) virus isolates collected, only three did not show resistance to adamantanes.[308] Patients that received oseltamivir treatment had a survival rate of 90.3%, and antiviral treatment was associated with a 20% reduced mortality risk when compared to no treatment.[309] In addition, patients receiving early oseltamivir treatment had shorter fever durations than patients who did not receive antiviral treatment.[310,311]

Antiviral treatment is also effective in reducing viral titer and duration of viral shedding in pH1N1 patients. According to one study, antiviral treatment reduced the mean viral load of patients by 14.3%.[312] On average, antiviral treatment reduced the duration of viral shedding in patients by 34% when compared to untreated patients or patients who received antiviral treatment after 48 hours, which has been shown to be less effective.[313]

A study in ferrets showed that prophylaxis with oseltamivir did not prevent H1N1 infection.[314] However, a household contact study showed that contacts under 20 years old who received antiviral prophylaxis with oseltamivir or zanamivir were nearly seven-fold less likely to be infected with pandemic H1N1 influenza than those who did not receive antiviral prophylaxis (odds ratio 0.15).[315] Additionally, other observational studies indicate that prophylaxis may be effective in preventing H1N1 infection.[316,317]

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307 McLean HQ *et al* (2015) Influenza vaccine effectiveness in the United States during 2012-2013: variable protection by age and virus type. *The Journal of infectious diseases* 211: 1529-1540

308 Gubareva LV *et al* (2010) Comprehensive assessment of 2009 pandemic influenza A (H1N1) virus drug susceptibility in vitro. *Antiviral therapy* 15: 1151-1159

309 Muthuri SG *et al* (2014) Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. *The Lancet Respiratory medicine* 2: 395-404

310 Yu H *et al* (2010) Effectiveness of oseltamivir on disease progression and viral RNA shedding in patients with mild pandemic 2009 influenza A H1N1: opportunistic retrospective study of medical charts in China. *BMJ* 341: c4779

311 Li IW *et al* (2010) The natural viral load profile of patients with pandemic 2009 influenza A(H1N1) and the effect of oseltamivir treatment. *Chest* 137: 759-768

312 Meschi S *et al* (2011) Duration of viral shedding in hospitalized patients infected with pandemic H1N1. *BMC infectious diseases* 11: 140

313 Nicholson KG *et al* (2000) Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. *Lancet* 355: 1845-1850

314 Oh DY *et al* (2014) Evaluation of oseltamivir prophylaxis regimens for reducing influenza virus infection, transmission and disease severity in a ferret model of household contact. *The Journal of antimicrobial chemotherapy* 69: 2458-2469

315 Odaira F *et al* (2009) Assessment of secondary attack rate and effectiveness of antiviral prophylaxis among household contacts in an influenza A(H1N1)v outbreak in Kobe, Japan, May-June 2009. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 14

316 Astedu-Bekoe F *et al* (2012) Mass oseltamivir prophylaxis halts pandemic influenza A H1N1 2009 outbreak in a secondary school in Ashanti Region, Ghana. *Ghana medical journal* 46: 219-224

317 Leung YH *et al* (2011) A school outbreak of pandemic (H1N1) 2009 infection: assessment of secondary household transmission and the protective role of oseltamivir. *Epidemiology and infection* 139: 41-44

Vaccines

Monovalent pH1N1 influenza vaccine demonstrated an average effectiveness of 66% (range 60-93%).[318,319]

5.4.14.4 Epidemiological Evidence of Effectiveness

The previous section describes the efficacy of vaccination in controlled, clinical trials. The epidemiological evidence is equivocal.

Supporting the benefit of vaccination, the Influenza Division at the CDC released a study on the mortality of the nine influenza seasons from 2005 to 2014. Their analysis found that approximately 22% of influenza-associated deaths were prevented by the influenza vaccine, with 90% of this benefit realized in persons over 65 years.[320] Further, they conservatively estimated 40,000 deaths to have been averted. The study found that the fewest deaths were prevented during the 2009– 2010 pandemic.[321] Another CDC study found that from 2005 to 2011, influenza-associated illnesses and hospitalizations were substantially alleviated by vaccinations. The analysis estimated that 1.1 to five million illnesses and 7,700 to 40,400 hospitalizations were prevented in those vaccinated against influenza. The study also found the largest benefit to be in elderly populations.[322]

Other studies found less favorable outcomes. Demicheli et al. presented a meta-analysis that showed poor effectiveness of the vaccine in reducing influenza cases and work days lost in healthy adults. The study did find, however, significant reductions in serologically confirmed cases of influenza.[323] According to the National Center for Health Statistics, while vaccination coverage spanned 15-20% of the elderly population by 1980 and increased to 65% in 2001, influenza-associated mortality substantially increased among those over 65 years old.[324] Likewise, a retrospective analysis covering the years 1979 to 2000 from The Institute for Chronic Illnesses found the vaccine to have little or no effectiveness for preventing influenza cases, deaths, or hospital admissions.[325] Simonsen et al. at the National Institute of Allergy and Infectious Diseases published that based on national vital statistics, other studies on the effectiveness of the influenza vaccine overestimate its benefits.[326] Despite increasing vaccination coverage from 1970 to 2001, Rizzo et al. found no evidence of a reduction in influenza-related morality in the Italian elderly population.[327] Several research groups propose that the overestimate of vaccine benefits is due to unrecognized confounding variables, such as a disproportionate amount of healthy elderly persons being

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318 Griffin MR *et al* (2011) Effectiveness of non-adjuvanted pandemic influenza A vaccines for preventing pandemic influenza acute respiratory illness visits in 4 U.S. communities. *PLoS One* 6: e23085

319 Osterholm MT *et al* (2012) Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *The Lancet Infectious diseases* 12: 36-44

320 Foppa IM *et al* (2015) Deaths averted by influenza vaccination in the U.S. during the seasons 2005/06 through 2013/14. *Vaccine* 33: 3003-3009

321 ibid.

322 Kostova D *et al* (2013) Influenza Illness and Hospitalizations Averted by Influenza Vaccination in the United States, 2005-2011. *PLoS One* 8: e66312

323 Demicheli V *et al* (2004) Vaccines for preventing influenza in healthy adults. *Cochrane Database Syst Rev*: CD001269

324 Simonsen L *et al* (2005) Impact of influenza vaccination on seasonal mortality in the US elderly population. *Arch Intern Med* 165: 265-272

325 Geier *et al* (2006) Influenza Vaccine: Review of Effectiveness of the U.S. Immunization Program, and Policy Considerations. *Journal of American Physicians and Surgeons*

326 Simonsen L *et al* (2005) Impact of influenza vaccination on seasonal mortality in the US elderly population. *Arch Intern Med* 165: 265-272

327 Rizzo C *et al* (2006) Influenza-related mortality in the Italian elderly: no decline associated with increasing vaccination coverage. *Vaccine* 24: 6468-6475

vaccinated.[328] Moreover, Madea et al., did not find significantly different levels of infection between vaccinated and unvaccinated healthy children six to 24 months of age.[329]

Regardless of differing opinions on vaccine efficacy, the decline in influenza morbidity and mortality over the past century is irrefutable. As Doshi et al. highlights, however, influenza vaccination was not available until the 1940s and not widely adopted until the 1980s and hence cannot be responsible for the drop (see Figures 5.11 and 5.12 above).[330]

Few studies exist on the relationship between influenza countermeasures and the decreases observed in morbidity and mortality, however the advent of antibiotics likely significantly reduced risk of death from influenza. In the absence of antibiotics, the majority of influenza mortality is attributed to interactions between the influenza virus and bacteria colonizing the upper respiratory tract, causing fatal secondary infections.[331] Evidence strongly suggests that the 1918 pandemic would have been greatly mitigated with the availability of antibiotics.[332]

5.4.14.5 Availability of Vaccines

Vaccine shortages have captured headlines several times over the past couple decades for various reasons, including underestimation of demand, reduction in manufacturers, contaminated issues, and unexpected outbreaks.[333] After the severe influenza vaccine shortages of the 2004 to 2005 season, the United States Government Accountability Office completed a study on the status of seasonal influenza preparation. The final report recognized that the vaccine shortage of 4.7 million doses, approximately half of the needed supply, exposed the need for better preparation for seasonal endemics.[334] During the 2009 H1N1 pandemic, experts predicted 160 million doses of the pandemic vaccine would be available for public vaccination by October, yet only 30 million were delivered by that date.[335]

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328 Osterholm MT *et al* (2012) Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *The Lancet Infectious diseases* 12: 36-44

329 Maeda T *et al* (2004) Failure of inactivated influenza A vaccine to protect healthy children aged 6-24 months. *Pediatrics international : official journal of the Japan Pediatric Society* 46: 122-125

330 Doshi P (2008) Trends in recorded influenza mortality: United States, 1900-2004. *Am J Public Health* 98: 939-945

331 Morens DM *et al* (2008) Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 198: 962-970

332 Doshi P (2008) Trends in recorded influenza mortality: United States, 1900-2004. *Am J Public Health* 98: 939-945

333 Tosh PK *et al* (2010) Influenza Vaccines: From Surveillance Through Production to Protection. *Mayo Clinic Proceedings* 85: 257-273

334 United States Government Accountability Office. Shortages in 2004-2005 season underscore need for better preparation (2005) Rep. No. GAL-05-984: 1-5

335 Vaccines Ho. Influenza Pandemics. http://www.historyofvaccines.org/content/articles/influenza-pandemics. Last Update July 2014. Accessed October 2015.

6 Risk Assessment of Laboratory Accidents and Natural Disasters

6.1 Overview of Results

The Biosafety Risk Assessment evaluated the increase in risk to human health of pandemics caused by modified strains of the influenza viruses and the coronaviruses. In every case, the increase in risk compared to wild type strains was provided to determine if GoF experiments could create pathogens that are *more likely* to cause laboratory acquired infections, *more likely* to create a local outbreak, or *more likely* to cause a global outbreak (or cause one of greater consequence) than those strains that evolved via natural forces. Note that although this study identified several types of risky, theoretical GoF experiments, many of these experiments have not been described in the literature. For example, no examples of researchers endeavoring to determine if seasonal influenza viruses could be made more transmissible were found. As another example, if a virus grows to a conveniently high titer naturally (e.g., 1E8 pfu/ml) then enhancing this level of growth may not be desirable or, indeed, biologically feasible. Moreover, many GoF studies are performed in highly attenuated strains, so that even though the risk of an outbreak increases if these strains were modified, risk is increasing from a very low level.

GoF Phenotype	Seasonal Influenza Viruses	1918 H1N1 Pandemic Influenza Virus	1957 H2N2 Pandemic Influenza Virus	Avian Influenza Viruses
Enhanced transmissibility	Increases probability of an outbreak and the consequences of an outbreak	Increases probability of an outbreak and the consequences of an outbreak		Increases probability of an outbreak and the consequences of an outbreak
Enhanced pathogenicity	Increases consequences		Increases consequences	
Adaptation to mammals	N/A	N/A	N/A	Decreases probability of an outbreak
Evasion of induced immunity	Increased consequences in high income countries only	Increased consequences in high income countries only	Increased consequences in high income countries only	
Evasion of natural/residual immunity	Increases probability of an outbreak and the consequences of an outbreak	Increases probability of an outbreak and the consequences of an outbreak		N/A
Antiviral resistance	Increased consequences in high income countries only	Increased consequences in high income countries only	Increased consequences in high income countries only	
Enhanced growth in culture/eggs		Increased chance of a LAI	Increased chance of a LAI	

Figure 6.1a. A figure showing increase in risk of research on modified influenza strains over wild type pathogens. The darker the shade of gray, the more a GoF phenotype increases risk of human illnesses and deaths. Marked in white are GoF phenotypes that are not relevant (N/A) to risk or reduce risk.

GoF Phenotype	MERS-CoV	SARS-CoV (R_0 1.6)	SARS-CoV (R_0 3.0)
Enhanced transmissibility	Increases probability of a global outbreak and consequences of a global outbreak	Increases probability of a global outbreak and consequences of a global outbreak	
Enhanced pathogenicity			
Adaptation to mammals	N/A	N/A	N/A
Evasion of induced immunity	N/A	N/A	N/A
Evasion of natural/residual immunity	N/A	N/A	N/A
Antiviral resistance	N/A	N/A	N/A
Enhanced growth in culture/eggs	Increased chance of a LAI	Increased chance of a LAI	Increased chance of a LAI

Figure 6.1b. A figure showing increase in risk of research on modified coronaviruses compared to wild type strains. The darker the shade of gray, the more a GoF phenotype increases risk of human illnesses and deaths. Marked in white are GoF phenotypes that are not relevant (N/A) to risk or reduce risk. This figure shows results assuming that the R_0 value of SARS-CoV is 1.6 (our baseline assumption) or 3.0.

In short, a strain of influenza virus that is as transmissible (or to which the population has as little minimal immunity) as newly emerged pandemic strains WHILE leading to a case fatality rate of more than 0.5% (the case fatality rate of the highly transmissible 1957 H2N2 pandemic strain) would pose more of a risk of a global pandemic than any wild type strain heretofore identified. No experiments that are likely to be conducted under the rubric of GoF research will drive risk more than this combination of traits or significantly increase the risk of a laboratory acquired infection. All other combinations of traits would lead to pathogens that have a lesser total risk than the wild type 1957 H2N2 strain. Increasing the transmissibility of the coronaviruses while significantly increasing the risk of work with those pathogens by several orders of magnitude still creates a pathogen that poses less of a risk of a global pandemic than the wild type 1957 H2N2 influenza strain.

In the brief section that follows, we provide the rationale behind these overall conclusions by showing how changes in each GoF phenotype affects each component of the risk assessment for each pathogen. Further supporting evidence is provided in this chapter in Section 6.4 and onward.

6.1.1 Seasonal Influenza Viruses

This risk assessment appropriately considers the fact that not all loss of containment events lead to a laboratory acquired infection, that not all laboratory acquired infections initiate a local outbreak (because of stochastic factors or the fact that infected workers may be given prophylaxis or be isolated), and that not all local outbreaks initiate a global pandemic. In fact, at each step, only a minority of events initiate the next step. Figure 6.2 shows the probability of each step in the chain of events that would eventually lead to a global pandemic from a loss of containment incident for wild type seasonal influenza, assuming that the previous step has occurred, assuming the work is conducted at BSL-2. From this figure, only 2% of laboratory acquired infections, which are rare in themselves (but not quantified by our method), start a local outbreak (that is, cause at least one secondary case) and only 20% of local outbreaks would seed a global pandemic. Moreover, in the case of seasonal influenza, the risk of a global pandemic is exacerbated by laboratory research only if that laboratory is working on a strain that has not circulated recently because residual immunity is likely to curtail its spread. If the strain is currently in circulation, the spread of the natural outbreak is likely to be driven by travelers, not by laboratory accidents.

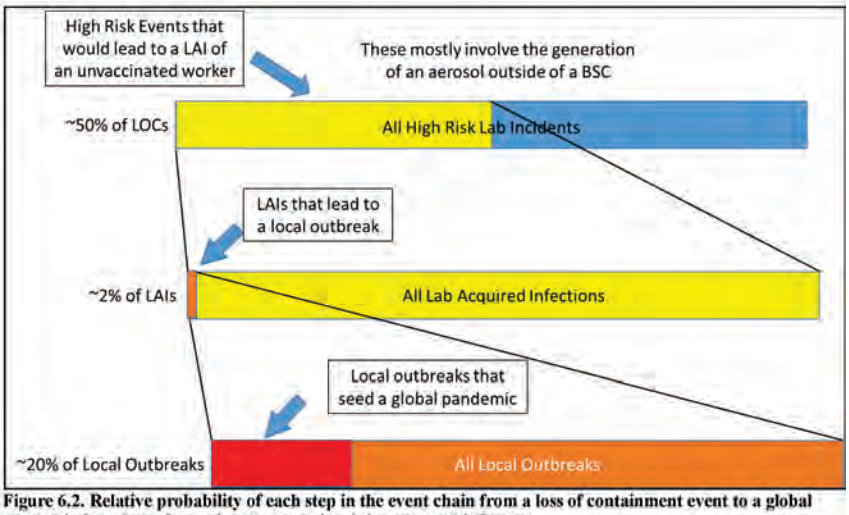

Figure 6.2. Relative probability of each step in the event chain from a loss of containment event to a global pandemic for a loss of containment event involving seasonal influenza.

To understand how GoF research could influence risk from research on seasonal influenza, it is useful to consider each step in the incident that leads from a loss of containment event to a global outbreak and to comprehend how the GoF trait would influence either probability or consequences at each step. Figure 6.3 divides risk by each step in the biosafety RA and shows how GoF research influences risk for seasonal influenza.

GoF Phenotype	Increase Probability of Lab Acquired Infection	Increase Probability of a Local Outbreak	Increase Probability an Outbreak Escapes Local Control	Increasing Global Consequences
Enhanced transmissibility	N/A	Less than 2x	2-3x	2x
Enhanced pathogenicity	N/A	N/A	Unknown—possible decrease in risk	10x or more
Adaptation to mammals	N/A	N/A	N/A	N/A
Evasion of induced immunity	Less than 2x	Less than 2x	N/A	4x in the high income countries only
Evasion of natural/residual immunity	Less than 2x	Less than 2x	2-3x	3-4x
Antiviral resistance	Less than 2x	Less than 2x	Unknown	5x in the high income countries only
Enhanced growth in culture/eggs	2x*	N/A	N/A	N/A

Figure 6.3. A figure showing increase in risk of GoF research on seasonal influenza over wild type seasonal influenza. The darker the shade of gray, the more a GoF phenotype increases risk of human illnesses and deaths. Marked in white are GoF phenotypes that are not relevant to risk (N/A), reduce risk or that could not be quantified. The star denotes a result that may not be statistically significant.

Because seasonal influenza viruses are associated with a low case fatality rate, increasing this rate could significantly increase the global death toll from an outbreak, increasing risk. Developing seasonal influenza strains that are more transmissible than wild type strains (approximately as transmissible as pandemic strains) or that overcome residual immunity increases the probability that an outbreak would escape local control and increases the consequences should a global outbreak be initiated. The creation of an antiviral resistant strain could increase the consequences of a global outbreak, but only in high income countries where caches of these antivirals could be handed out to a significant fraction of the infected population.

A strain of seasonal influenza that can overcome protective vaccination could also increase the consequences of an outbreak in high income countries, which has the resources to vaccinate their populations quickly. However, this phenotype is of concern only if it enables the virus to evade the protection afforded by means other than changing its antigenic properties, which is not a subject of current research in influenza.[336] (The vaccines made in response to an outbreak caused by a laboratory accident would be raised specifically against the strain causing the outbreak, so it would "match" the

[336] Clearly, this phenyotype increases risk given that enough vaccine could be produced in a short enough time to influence the outbreak caused by wild type strains.

novel antigenic properties of the strain.) The relatively small increase in risk due to vaccine resistance is not unexpected due to the modest efficacy of seasonal influenza vaccine at preventing infection.[337]

6.1.2 Pandemic Influenza Viruses

Figure 6.4 shows the relative probability of each step in the chain of events that would eventually lead to a global pandemic from a loss of containment incident for pandemic influenza at BSL-3. From this figure, less than 1% of high-risk loss of containment events, which are rare in themselves but not quantified here, involving wild type pandemic influenza would lead to a laboratory associated infection; only 5% of laboratory acquired infections start a local outbreak and only 20% of local outbreaks seed a global pandemic.

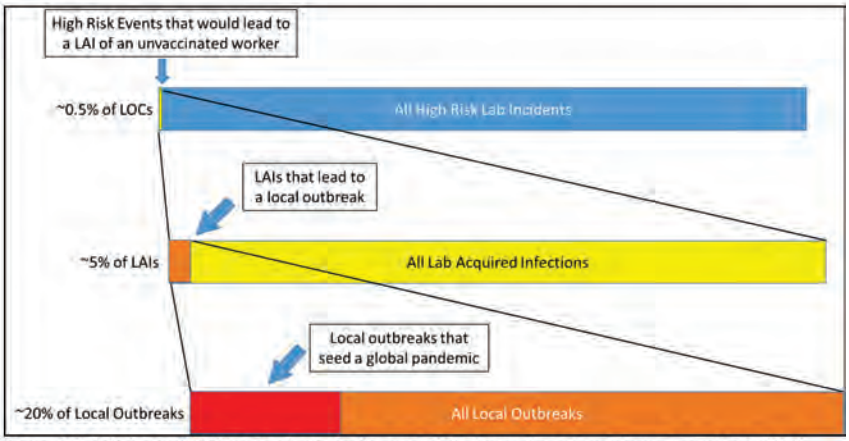

Figure 6.4. Relative probability of each step in the event chain from a loss of containment event to a global pandemic for a loss of containment event involving pandemic influenza.

Figure 6.5 divides risk by each step in the biosafety RA and shows how GoF research influences risk for pandemic influenza, assuming the strain manipulated is 1918 H1N1 influenza or 1957 H2N2 influenza. The risk analysis suggests that only two lines of GoF research could create a strain of pandemic influenza that poses more risk of a global outbreak than a wild type strain (in this case, the 1957 H2N2 pandemic strain). The first is the manipulation of a strain of 1918 H1N1 pandemic influenza that is modified to evade residual immunity (or otherwise increase transmissibility to the same degree). The second is the enhancement of pathogenicity (to that of 1918 H1N1 influenza) of a highly transmissible pandemic strain (e.g., 1957 H2N2 influenza). Imbuing 1957 H1N1 influenza with antiviral resistance can modestly increase the consequences of an outbreak, but only in countries with significant caches of antivirals. Enhancing viral growth in culture beyond that which is achievable in wild type strains (1E9 or 1E10/ml) increases the probability that a laboratory acquired infection would occur (by five- or 15-fold, respectively). However, it is doubtful if this phenotype is desirable or scientifically achievable because growth to 1E8 is sufficient for almost all purposes except the production of vaccines (using attenuated strains).

[337] Cowling, BJ et al "Assessment of influenza vaccine effectiveness in a sentinel surveillance network 2010-13, United States." *Vaccine* 2015, article in press.

GoF Phenotype	Increase Probability of Lab Acquired Infection	Increase Probability of a Local Outbreak	Increase Probability an Outbreak Escapes Local Control	Increasing Global Consequences
Enhanced transmissibility	N/A	Less than 2x	Up to 3x increase	100X or more increase
Enhanced pathogenicity	N/A	N/A	Unknown—possible decrease in risk	Less than 2x
Adaptation to mammals	N/A	N/A	N/A	N/A
Evasion of induced immunity	Less than 2x	Less than 2x	N/A	Up to 4x increase in high income countries only
Evasion of natural/residual immunity	Less than 2x	Less than 2x	Up to 3x increase	100x or more increase
Antiviral resistance	Less than 2x	Less than 2x	Unknown	Up to 8x increase in high income countries only
Enhanced growth in culture/eggs	Up to 6x	N/A	N/A	N/A

Figure 6.5a. A figure showing increase in risk of GoF research on 1918 H1N1 pandemic influenza over wild type 1918 H1N1 pandemic influenza. The darker the shade of gray, the more a GoF phenotype increases risk of human illnesses and deaths. Marked in white are GoF phenotypes that are not relevant to risk (N/A), reduce risk or that could not be quantified.

GoF Phenotype	Increase Probability of Lab Acquired Infection	Increase Probability of a Local Outbreak	Increase Probability an Outbreak Escapes Local Control	Increasing Global Consequences
Enhanced transmissibility	N/A	Less than 2x	Less than 2x	Less than 2x
Enhanced pathogenicity	N/A	N/A	Unknown—possible decrease in risk	Up to 10x increase
Adaptation to mammals	N/A	N/A	N/A	N/A
Evasion of induced immunity	Less than 2x	Less than 2x	N/A	Less than 2x
Evasion of natural/residual immunity	Less than 2x	Less than 2x	Less than 2x	Less than 2x
Antiviral resistance	Less than 2x	Less than 2x	Unknown	2-3x increase in high income countries only
Enhanced growth in culture/eggs	Up to 6x	N/A	N/A	N/A

Figure 6.5b. A figure showing increase in risk of GoF research on 1957 H2N2 pandemic influenza over wild type 1957 H2N2 pandemic influenza. The darker the shade of gray, the more a GoF phenotype increases risk of human illnesses and deaths. Marked in white are GoF phenotypes that are not relevant to risk (N/A), reduce risk or that could not be quantified.

6.1.3 Avian Influenza Viruses

Wild type avian influenza is insufficiently transmissible amongst people to cause a global outbreak driven by the spread of the disease among humans. For this reason, no loss of containment event would lead to a global outbreak from a wild type strain.

Figure 6.6 divides risk by each step in the biosafety RA and shows how GoF research influences risk for avian influenza. Because wild type strains of avian influenza cannot spread globally between people, the creation of strains that are human transmissible would greatly increase the risk that such an outbreak could occur, which could cause millions of illnesses. The creation of a strain that is as transmissible as seasonal influenza would have a significant chance of sparking a global outbreak if a local outbreak were initiated. An increase in the pathogenicity in humans of the most pathogenic, wild type strains increases the consequences modestly only if one assumes that the case fatality rates of the most pathogenic strains of avian influenza are inflated by the underreporting of mild illness in people. Adapting avian strains to humans without increasing transmissibility (thereby lowering the median infectious dose in people) actually decreases risk because, while this trait increases the probability that a single laboratory worker would become infected, it decreases the risk that birds would become infected through an accidental release via the solid waste stream, which otherwise could lead to thousands of human infections and is the dominant loss of containment pathway. No other GoF trait increases the risk posed by avian influenza.

GoF Phenotype	Increase Probability of Lab Acquired Infection	Increase Probability of a Local Outbreak	Increase Probability an Outbreak Escapes Local Control	Increasing Global Consequences
Enhanced transmissibility	Less than 2x	Wild type strains cannot cause a local outbreak	Wild type strains cannot escape local control	Wild type strains cannot cause a global outbreak of human disease
Enhanced pathogenicity	N/A	N/A	N/A	Less than 2x
Adaptation to mammals	Decrease in risk	N/A	N/A	N/A
Evasion of induced immunity	Less than 2x	Less than 2x	N/A	Less than 2x
Evasion of natural/residual immunity	N/A	N/A	N/A	N/A
Antiviral resistance	Less than 2x	Less than 2x	N/A	Less than 2x
Enhanced growth in culture/eggs	Less than 2x	N/A	N/A	N/A

Figure 6.6. A figure showing increase in risk of GoF research on avian influenza over wild type avian influenza. The darker the shade of gray, the more a GoF phenotype increases risk of human illnesses and deaths. A numerical value cannot be provided for the greatest increases because the risk from wild type pathogens is vanishingly low for these outcomes. Marked in white are GoF phenotypes that are not relevant to risk (N/A) or reduce risk.

6.1.4 Coronaviruses

Figure 6.7 divides risk by each step in the biosafety RA and shows how GoF research influences risk for the coronaviruses. Recall that the RA uses the word "coronavirus" to mean the coronaviruses that cause SARS or MERS and not the coronaviruses that cause the common cold. Importantly, most estimates of the transmissibility of the coronaviruses consider these pathogens to be insufficiently transmissible and sufficiently susceptible to control measures such that a global pandemic has a very minimal chance of occurring. Even using the highest estimates of R_0 for SARS-CoV, derived from the location and time of an outbreak that caused the most secondary infections, results in less than a 10% chance of sparking a global pandemic should a local outbreak begin. For this reason, increasing the transmissibility of the coronaviruses could significantly increase the chance of a global pandemic due to a laboratory accident. That being said, even if these strains were modified to be as transmissible as pandemic influenza, the viruses' long generation time and lack of asymptomatic transmission, which results in susceptibility to control measures, the resulting outbreaks would still be contained a majority of the times they were initiated. Some researchers, using the strictest definition of R_0, have calculated the R_0 of SARS-CoV to be

3.0.[338,339] If SARS-CoV is indeed this transmissible, than the probability of escape or the consequences of a global outbreak are not increased significantly by further increases in transmissibility.

Increasing the pathogenicity of these strains could also increase risk somewhat through the increase in global deaths expected, considering most deaths from wild type strains are suffered by those with significant co-morbidities. Strains of the coronaviruses that have enhanced growth properties could increase risk of a laboratory acquired infection if samples with 1E9pfu/ml or 1E10pfu/ml were routinely manipulated in a laboratory (risk would increase by seven- or 25-fold, respectively). However, it is uncertain if this phenotype is desirable or even achievable because wild type coronaviruses grow to a sufficiently high titer for manipulations in the laboratory.

GoF Phenotype	Increase Probability of Lab Acquired Infection	Increase Probability of a Local Outbreak	Increase Probability an Outbreak Escapes Local Control	Increasing Global Consequences
Enhanced transmissibility	N/A	Less than 2x increase	Wild type strains are highly susceptible to local control	Several orders of magnitude
Enhanced pathogenicity	N/A	N/A		2-3x increase
Adaptation to mammals	N/A	N/A	N/A	N/A
Evasion of induced immunity	N/A	N/A	N/A	N/A
Evasion of natural/residual immunity	N/A	N/A	N/A	N/A
Antiviral resistance	N/A	N/A	N/A	N/A
Enhanced growth in culture/eggs	Up to a 10-fold increase in probability for a 1E10pfu/ml culture	N/A	N/A	N/A

Figure 6.7. A figure showing increase in risk of GoF research on coronaviruses over wild type coronaviruses. The darker the shade of gray, the more a GoF phenotype increases risk of human illnesses and deaths. A numerical value cannot be provided for the greatest increases because the risk from wild type pathogens is vanishingly low for these outcomes. Marked in white are GoF phenotypes that are not currently relevant to risk (N/A).

However, if a coronavirus were modified such that it caused a global pandemic (one in which sustained human-based transmission occurs in all global regions, which has never been observed), their relatively long incubation time and disease course (compared to influenza) would lead to a pandemic that unfolds over many years (Figure 6.8). While some outbreaks peak within two years, most require two to ten years to reach their peak. The fact that the outbreak evolves slowly gives public health authorities more time to adapt and expand their efforts to further contain the outbreak than the modeling conducted in this assessment suggests.

[338] Lipsitch, M., et al., Transmission dynamics and control of severe acute respiratory syndrome. Science, 2003. 300(5627): p. 1966-70.
[339] Wallinga, J. and P. Teunis, Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures. Am J Epidemiol, 2004. 160(6): p. 509-16.

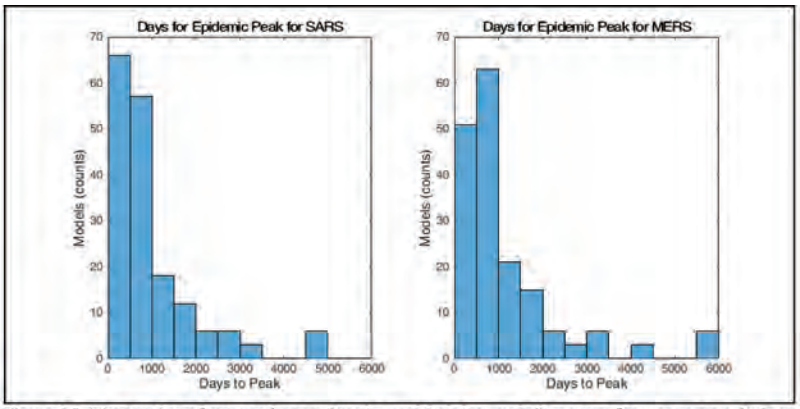

Figure 6.8. The number of coronavirus outbreaks modeled that peak (in terms of new cases per day) at any particular day after the global outbreak begins. To show the duration of truly global outbreaks, outbreaks that lead to less than one million infections are not shown.

6.2 Methodology

6.2.1 Purpose of This Task

The purpose of the quantitative biosafety RA is to provide information regarding the risk (in terms of consequences and probability) of a release of strains of pathogens with novel phenotypes that enhance their pandemic potential due to an accident or natural disaster. This assessment has three main components:

1. The estimate of the probability that an accident/natural disaster would occur and result in an infection of a human or other animal outside the laboratory,
2. The estimate of the probability that an outbreak that occurs would escape beyond local control and seed a global outbreak, and
3. The estimate of the extent of an outbreak that would result from an infection outside the laboratory.

Critically, because GoF research occurs in a world in which research on dangerous, wild type pathogens is ongoing, the risk assessment is comparative. That is, we seek to determine how much risk *increases* if GoF research proceeds.

6.2.2 Input from Modeling Subject Matter Experts

To guide our modeling effort, we interviewed the following infectious disease modeling subject matter experts (SMEs): Dr. Jason Asher, Dr. Steven Riley, Dr. Martin Meltzer and Dr. Carrie Manore.[340,341,342,343] Their input is reflected in the modeling methodology described in this section. All of the interviewed experts unanimously agreed that the use of multiple models, covering event initiation, initial local spread, and potential global outbreak, respectively, is reasonable and sound. Additionally, all experts confirmed that the choices of a stochastic approach for the initiation phase of an outbreak followed by a homogenous mixing, deterministic approach for modeling the global spread phase were appropriate. Mr. Asher spoke about the BARDA Interactive Flu Model, an SEIR-type model, and confirmed that it contained the necessary features for the biosafety risk analysis; this model became the basis for global outbreak simulations.

When asked about appropriate stochastic models for the initiation phase of the outbreak, Dr. Riley suggested that, in lieu of a computationally-intensive agent based model, a branching process model may be more appropriate. He believed that such an approach would capture the key features of such an outbreak, while leaving out dimensions that were not critical in determining whether an outbreak would grow beyond local control, such as where infected individuals lived. He also remarked that branching process models would capture a critical facet of laboratory acquired infections: that most of them do not lead to outbreaks of significant size. Moreover, an agent-based model would require the parameterization of features of the environment of the outbreak that would be unknowable. Dr. Riley emphasized the criticality of the shape of the offspring distribution in such a model and suggested we speak with Dr. James Lloyd-Smith, with whom Gryphon collaborated and consulted on the development of the branching process model used in the final risk analysis.[344] All other interviewees to whom a branching process model was mentioned either raised no objections or confirmed the appropriateness of the approach.

In searches of the literature, little data and few models covering zoonotic infections of influenza were found. Dr. Manore agreed that relatively little data existed, particularly for interspecies contact rates, and that few people were considering models that incorporated humans, domestic animals, and wildlife in a single model. She remarked that their approach to overcoming this lack of data in their influenza models was to parameterize based on a retrospective analysis of prior outbreaks to ensure that the predictions of the model were reasonable. This approach is clearly not suitable for a prospective analysis such as ours.

6.2.3 Interplay of the Components of the Biosafety Risk Assessment

The biosafety RA has several components that will be married together to understand how risk of a laboratory accident changes if GoF experiments proceed. These components and their interplay are shown in Figure 6.9.

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340 Leidos contract support to the Division of Analytic Decision Support, Biomedical Advanced Research and Development Authority, Department of Health and Human Services, Washington, United States

341 MRC Centre for Outbreak Analysis and Disease Modelling, Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, United Kingdom

342 National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control, Atlanta, GA, United States

343 Center for Computational Science, Tulane University, New Orleans, LA, United States

344 Department of Ecology and Evolutionary Biology, University of California, Los Angeles, California, United States of America

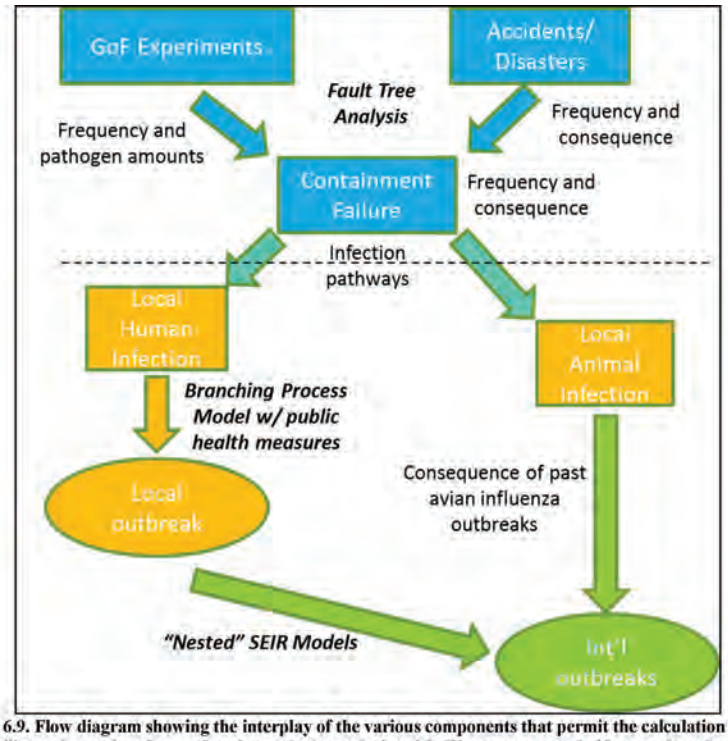

Figure 6.9. Flow diagram showing the interplay of the various components that permit the calculation of the probability and severity of an outbreak to calculate relative risk. The components in blue speak to the probability that an infection outside the laboratory occurs. The components in orange speak to the probability that an outbreak will escape local control and cause a global pandemic. The components in green speak to the consequences of a global outbreak. Quantitative modeling approaches are shown in bold italics. All components are considered together to understand probability and consequence using a Probabilistic Risk Assessment framework.

To inform the RA, we interviewed 77 Subject Matter Experts as shown in Figure 6.10. These stakeholders provided data on the frequency of GoF experiments and the experimental conditions, containment features, health surveillance procedures, isolation procedures, and public health response measures that occur in their laboratories.

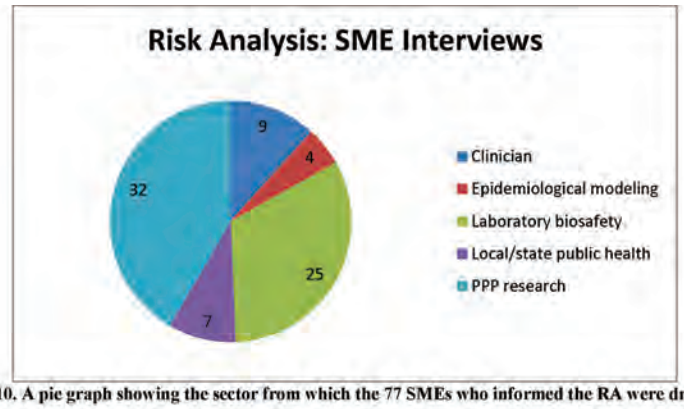

Figure 6.10. A pie graph showing the sector from which the 77 SMEs who informed the RA were drawn.

6.2.4 Taking a Parametric Approach to the Analysis

Perhaps the most challenging aspect of this effort is to assess the risk of experiments that have yet to occur in locations that haven't been identified performed under unknown biosafety conditions. If there were a finite number of phenotypic changes to the pathogens of interest, a finite number of experimental procedures used, a small number of possible locations for the research or a finite number of conditions under which the research could occur, we could simply test each one. However, this approach is not feasible and would not provide the NSABB with the information it needs to make recommendations and for the USG to formulate policy.

Worse still, drawing a "bright-line" boundary between research that qualifies as GoF and research that does not is likely to be difficult. Even for experiments of the same type, the specific strains selected, the quantities used, and the intended outcomes matter greatly; the context of the research is key. As a result, without a method that allows an examination of the details of each experiment and the pathogens therein, accurately assessing the relative risks of each would be difficult, if not impossible.

For this reason, the power of the modeling approach was exploited to explore the entire space describing each quality of GoF and wild type pathogens used, the experiments undertaken, the laboratories, and the containment measures where the experiments occur. This type of approach is called "parametric analysis" because each quality, or parameter, is allowed to vary across a range of possible values. This approach enabled not only the accurate assessment of the risk of future experiments, but also can be used to support the development of generalizable guidelines after important drivers of risk are identified.

This approach was applied to the pathogens themselves, for which the following parameters were allowed to vary, including:

- Pathogenicity in humans (including case fatality rate and infectious dose),
- Transmissibility in humans,
- Evasion of diagnostics and countermeasures, including vaccines and antivirals,
- Evasion of immunity, either natural or induced, and
- Growth in culture or eggs

The approach was applied to the experimental conditions, for which the following parameters were allowed to vary, including:

- The number and type of infected animals,
- The frequency animals are physically handled,
- The concentration and volumes of stocks and infected tissue culture samples, and

Although we considered pathogens with a range of characteristics, any of these pathogens were considered to have arisen from an already established strain of virus in terms of the experiments conducted and epidemiology. Moreover, we specifically parameterized and modeled wild type influenza viruses (seasonal, pandemic and avian), MERS Co-V, SARS Co-V (to establish the baseline for relative risk), and the strains that have arisen from GoF experiments already (to identify any change in risk).

This approach was applied to the laboratories that may perform the work, for which the following parameters were allowed to vary, including (a full list is available in the Supplemental Information):

- The existence and effectiveness of various containment features,
- The existence and effectiveness of various pieces of personal protective equipment (PPE),
- The existence and effectiveness of decontamination procedures,
- The existence and effectiveness of monitoring systems for the health of the workforce, and
- The population density of humans outside the laboratory,

To understand risk of GoF work when performed under less-than-ideal circumstances, as may be the case in other parts of the world, we can assess how the removal of any particular containment feature, decontamination procedure, or health monitoring procedure would affect the probability of a release.

6.2.4.1 Bounding the Parametric Approach in Science

The parametric analysis described above is also underpinned by scientific data. That is, a parameter could be allowed to vary between any arbitrarily large or small value but is grounded by information related to the attributes of known pathogens. For example, the contagiousness (which could be measured in the number of naive people an infected person can infect) could be allowed to vary from zero to the entire population of the earth. However, given data on real viruses, we know that this parameter value would rarely exceed ten and only when describing the most contagious viruses known. Moreover, the possibility that a modified avian influenza virus becomes *more* contagious than any influenza virus that has ever been observed stretches reason. Similarly, even though the parametric analysis will allow the systematic removal of containment features, it is highly unlikely that GoF experiments would proceed without any containment whatsoever. As another example, an extremely risky strain of influenza virus may be one that could be simultaneously transmissible in poultry and humans, however such a strain may not be possible due to the nature of the changes in viral receptors that lead to changes in species tropism.

Our parametric analysis allowed us to evaluate how risk changes as the GoF research features change. The possibility that risk increases significantly only when a parameter reaches an unrealistic value builds confidence in the fact that our model is capturing all possible facets of risk. At the same time, this counterfactual analysis provides some information as to which manipulations or settings are unlikely to pose a significant increase in risk over work with unmodified pathogens.

Although the intent was to develop an RBA approach that is flexible enough to encompass all possibly risky manipulations of any pathogen, many aspects of a pathogen's lifecycle (and its epidemiology) are unique, and therefore models that faithfully replicate the risk of outbreak initiation and spread must use

real examples. Moreover, the experimental conditions used in GoF experiments (volumes, titers, cell lines, animal models, etc.) are also unique to the pathogens used in the experiments. Due to the focus of GoF concerns on influenza and coronaviruses, we used these viruses as the basis of our work in this study.

6.2.5 Probability of an Infection Outside of Containment

6.2.5.1 Choosing the Incidents to Model in Detail

To estimate the probability that an accident or natural disaster (together called incidents) leads to an infection outside the laboratory, we treated each separately. There are several types of incidents that could cause a loss of containment and a subsequent outbreak outside a containment laboratory. To identify the incidents to model, we leveraged previous laboratory risk studies and reports on past incidents to understand which incidents most drive risk of outbreaks caused by incidents at containment laboratories. Minor accidents, which do not drive risk because they were found to be unlikely to cause an infection or to have minimal consequences should they occur, need not be considered in detail. Recall that because risk is the product of the consequence and frequency of an adverse event, the riskiest accidents to examine include a variety of types: 1) those that are frequent and low-consequence, 2) those that are rare and high-consequence, and 3) those that are not uncommon and of moderate consequence. After the identification of the incidents that drive risk, the remainder of the biosafety RA analysis focuses on the evidence basis and modeling of only these most risky accidents. We further winnowed out incidents that were found to be minimally risky in the context of GoF research.

We collected past laboratory RAs and Environmental Impact Assessments (listed in Appendix III). For all studies that quantitatively assess risk in terms of probability and consequence, we identified the highest risk incidents and gathered all data related to those incidents. For studies that simply detail scenarios that are deemed to be "maximum reasonably foreseeable events" or a "plausible, worst case" scenario, we determined if quantitative studies examined similar incidents and where they fall on the risk ranking. As these types of scenarios are typically chosen because they have maximal consequences, without a consideration of probability of the event occurring, it is possible that these so-called "maximum reasonably foreseeable events" or "plausible, worst-case" scenarios are so vanishingly unlikely (i.e., occurring less than once in a billion years) that they do not affect risk much, even though they are consequential when they occur. If these scenarios were found to be relatively low risk, they were excluded from further analysis. If they were assessed in other quantitative risk assessments or there was no other reason to exclude them, they were included in our Fault Tree Analysis. This process explicitly captures the low-probability (but plausible), high-consequence events.

To supplement our list of high-risk accidents from previous assessments, we examined accident reports and case studies (sources are listed in Appendix III). Importantly, historical incidents are supported by a minimal amount of quantitative information (mostly related to consequence) that prohibits an estimate of risk. Just because an accident occurred once, we cannot calculate the probability that it would happen again. For this reason, we compared the list of historical accidents to the list of incidents to model from past RAs. Also, we found that many incident reports are included as high-risk incidents in past RAs (for instance, "spill") and other incidents are components of an overall sequence of failures that leads to a release in a past RA. For example, "PPE failure", which is mentioned in accident reports, is a possible failure node in all incidents that involve the generation aerosols or splashes on personnel. We included these types of events in several fault trees to assess the influence of the failure of these systems on risk of another incident (such as a spill). From these riskiest accidents, we removed those accidents that do not apply to the pathogens we are considering. For example, the National Bio- and Agro-defense Facility Site Specific Risk Assessment conducted for the Department of Homeland Security in 2012 identifies several

risky accidents arising from the fact that their pathogens are studied in large animals (like cattle), which can physically break containment features.

The list from previous studies and reports consists of the riskiest incidents that cannot be discounted from previous studies, the most common accidents that could lead to an infection outside a laboratory, any accident that did lead to an infection outside the laboratory (that cannot be discounted), and all "maximum reasonably foreseeable events" that could not be shown to be lower in risk than incidents included.

Even though the highest risk accidents are unlikely to change much in their frequency regardless of the nature of the pathogen, the probability that an infection outside the laboratory could occur may be significantly different. That is, although the chance that a centrifuge rotor breaks is the same if the sample inside contains viruses or bacteria, the chance that an infection may occur outside the laboratory if a worker caries infected material out on his shoe may be different if the contaminant were Foot and Mouth Disease virus versus influenza virus. For this reason, we determined if the pathogens of particular interest in GoF studies, specifically influenza viruses, MERS-CoV, and SARS-CoV, pose unique risk pathways that must be investigated further due to dissimilarities of their biology, pathogenesis, host range, or life cycle compared to the pathogens considered in past RAs. From this qualitative analysis, we identified further accidents to consider to capture the unique risks that these pathogens may pose. Specifically, animal bites were a "low risk" incident in past RAs, but ferrets, an animal model of choice in influenza studies, are particularly prone to biting (which, although the risk of infection from the bite is unlikely for respiratory pathogens, could deposit pathogen directly on the skin, increasing the risk of self-inoculation into the eye or nose). An "incident" modeling the fact that infected animals are constantly exhaling pathogen (called "animal respiration") was also specifically included because, unlike in other laboratories, infected animals pose a direct hazard to unprotected workers (should containment fail). All highest risk, relevant incidents from past studies and case reports were combined with these additional selected incidents to define the list of high-risk incidents that we were investigated in detail.

6.2.5.2 Predicting the Amounts and Pathways of Pathogen Releases for Accidents

To assess the probability that an accident would occur resulting in a loss of containment, we used Fault Tree Analysis (FTA), an accident modeling approach in which each possible system component that could fail in a complex pathway to an accident is explicitly parameterized with probability and consequences of failure. We implemented this FTA using Monte Carlo simulations, an approach that randomly samples values from all possible parameter values to explore the effect of uncertainty on our analysis. This approach was used to determine the probability that an accident occurs while an experiment with a dangerous pathogen is taking place (or in the handling or shipment of a pathogen) and the amount of material that escapes containment. Should an accident occur, there will be consequences in terms of the material dispersion. The dispersed material will then be subject to elimination or retention due to decontamination procedures and containment systems. The fact that many accidents may not be apparent when they occur is important to consider because additional measures are usually implemented when overt accidents occur. The accident could generate an infectious aerosol, fomites, or living carriers (laboratory animals or workers). We also considered the possibility that an accident generates many types of sources (a centrifuge spill could create an aerosol, fomites, and infected workers).

For accidents, the frequency of experiments and the concentration of the stocks and samples manipulated were estimated to describe the "opportunity space" for accidents to befall. These data were gathered in site visits and interviews with PPP researchers. Once an accident occurs, the agent may be released but will still be inside of containment. The effectiveness of containment measures determines how much material leaves containment depending on the nature of the accident. Containment measures reduce the concentration of a biological release, but may not be in place/functioning where the accident occurs due

to human error (e.g., mislabeling/mishandling of a sample) or equipment failure. Data on the failure of mechanical systems and of human error rates were derived from the scientific/engineering literature. Data supporting the Fault Tree Analysis are provided in the Supplemental Information along with full descriptions of the Fault Trees. Given the role of humans in historical laboratory accidents, our FTA includes a robust consideration of human reliability in the execution of appropriate decontamination and safety procedures.

Any material that escapes containment and decontamination described a source term that is used to model the initiation of an infection outside the laboratory. Aerosols were described by their quantity in a respirable range. Fomites were described by their material, location, and quantity. Infected people and animals were described by their type and quantity. Insofar as an accident causes consequences inside the laboratory to the workers, these casualties were tallied as consequences.

6.2.5.3 Source Terms for Natural Disasters

For natural disasters, we estimated, at any given time, how much pathogenic material is in the laboratory that could be released. This pathogenic material could be in the form of stocks in storage, samples being manipulated, or infected animals. The disaster itself may lead to several events inside the laboratory (the spill of materials or the release of animals) and the disruption of containment systems (over pressuring of HEPA systems or breach/failure of a building envelope). Several infection pathways could simultaneously lead to outside infection after a natural disaster (an earthquake could lead to the generation of an agent aerosol and the escape of infected animals). FTA was used to determine the probability that a natural disaster occurred and affected pathogen stocks, infected animals, or experiments in progress. As described above, we examined only those natural disasters that are deemed to be high risk. The dispersed material will then be subject to elimination or retention due to decontamination procedures and containment systems, although these may be compromised due to the disaster. Natural disasters cannot be covert, and so we assume that special public health measures (such as social distancing or restrictions on movement) would be implemented if a natural disaster is known to strike a containment laboratory.

Any material that escapes containment and decontamination helps describe a source term that was modeled for its ability to cause an infection outside of a laboratory. The source terms were described similarly to those arising from accidents.

6.2.5.4 Modeling Initiation of an Infection Outside a Laboratory

Once infected material leaves the laboratory, it may cause infections in nearby human or animal populations. The probability of the infection occurring depends on the nature of the source term, which can be aerosols, fomites, or infected animals/researchers. Each type of source term was modeled using a separate methodology.

Indoor and outdoor source terms were modeled separately. Indoor source terms were modeled as if the worker causing the accident inhaled all of the aerosol to understand a maximum level of risk. We chose this approach because a worker who creates an aerosol is exposed to a relatively high concentration of the contaminant until it disperses within the room. Workers who did not create the aerosol are exposed by dwelling in a completely well-mixed space that is slowly exhausted to the outside. Any material escaping the building was modeled using the Hazard Prediction and Analysis Capability (HPAC), a model developed by the Defense Threat Reduction Agency which is able to predict the transport and downwind infections over large areas given real population densities. We chose two laboratory locations to understand the range of risk from aerosols, New York City—the urban area with the greatest population density in the US— and a small town. Real weather data for those locations was used. One hundred

releases were modeled ranging over a variety of times of year and times during the work day. HPAC was used to calculate the dose that people downwind received. Dose/response curves were used to determine how much of the population inhaling the pathogen becomes infected. Given data on the populations of susceptible animals (specifically ducks) and their minute tidal volume (the amount of air they inhale per minute), we calculated animal infections over the same area.

For infected animals that leave the laboratory due to a natural disaster, we presumed that an outbreak is initiated by the animal encountering a human before it expires (for human-transmissible pathogens) or by encountering a susceptible bird (for bird-transmissible pathogens). For infected animals that leave the laboratory because they are carried out intentionally in a malicious act (relevant to the biosecurity RA below), we presumed that the malicious actors are themselves infected (for human-transmissible pathogens) or that the infected animal encounters a susceptible bird (for bird-transmissible pathogens). For the animal escape incident in the biosafety RA, our FTA models predict the animal leaving the laboratory is vanishingly unlikely (by bolting, unnoticed through several self-closing doors) and instead drives risk by escaping containment features within the lab and infecting workers.

For infected workers, we created a separate FTA that accounts for their behavior, the possible violation of health monitoring procedures and isolation guidance and their contacts with susceptible individuals throughout their disease course. Some protocols are initiated only if the exposure event was overt and considered high risk (an observed spill for example). Other protocols, such as the reporting of influenza-like illness and isolation should such symptoms appear, occur regardless of the type of accident that caused the illness. Workers may violate protocols (via ignorance or arrogance), and these workers enter into the models of local infection, as described below. Also, a worker could initiate a local outbreak if they develop no clinical symptoms or develop transmissible illness prior to the onset of symptoms.

For fomites, we developed a stochastic, Markov chain model to predict the likelihood of an outbreak initiating after a laboratory worker leaves containment with virus on his or her person. The model tracks the contamination through the paths it must take to result in infection of the initial laboratorian, of one or more household or community members, or of avian species on a farm (or any combination of the three). All infections are the result of internalization of the virus from a contaminated surface or body part; that is, this is a model of contamination transference and subsequent infection, not a model of contagious transmission. The transference model utilizes Monte Carlo simulations to estimate the likelihoods of a number of possible actions that would lead to internalization, spread, or removal of the virus. Human infection occurs when viral contamination on a person's hand enters their mucosal membranes of the eye, nose, or mouth, and the probability of infection is dose-dependent based on the calculated amount of virus present at the time of inoculation. For an animal infection to occur, the primary laboratorian must encounter a susceptible species, at which point it is assumed that all of the virus is inoculated into the animal. For animal contact to occur, the worker may need to violate quarantine protocol, which occurs at a specific probability, after which visits to an animal facility occur at a predetermined rate, as with the events above.

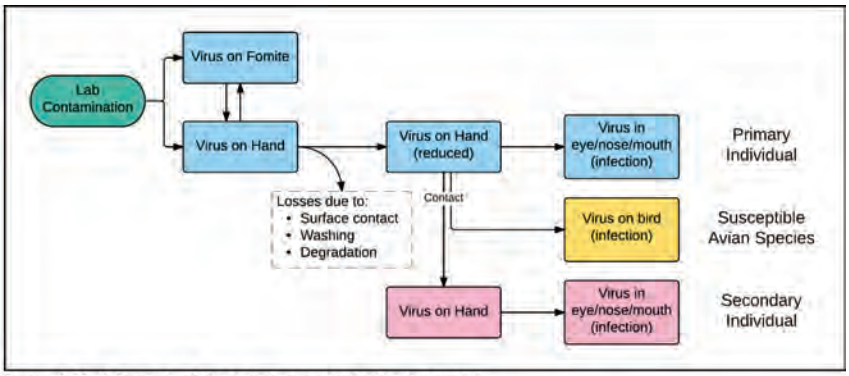

Figure 6.11. Schematic of the transference and infection model.

For the data supporting the parameter values used in the models that support the estimate of source terms causing infections outside the laboratory, please see the Supporting Information.

This analysis predicts the probability that an outbreak would be initiated by human or avian infections outside the laboratory for each of the possible incidents modeled. These incidents can vary by the species infected (ducks or people), the type of person infected (laboratory worker or a member of the public) and the number of people infected.

6.2.6 Predicting the Probability That an Outbreak Escapes Local Control

Once a human-transmissible disease leaves the laboratory and infects at least one person outside of containment, an outbreak is initiated (recall that health monitoring and isolation/quarantine measures enacted for exposed laboratory workers are already considered before the outbreak occurs, as described above). Depending on the release, an outbreak can start with one or more initial cases. For example, a large aerosol release from a catastrophic incident (like an earthquake) could infect many dozens of people. We considered outbreaks that initiate with the infection of a laboratory worker differently than outbreaks that begin with a member of the public because we presume that laboratory workers (and their families) would be more likely to report to public health authorities if they developed unusual symptoms of infectious disease and would be more likely to self-isolate.

An outbreak that starts with a handful of people is governed by stochastic forces that could, by chance, cause the outbreak to extinguish. Similarly, an outbreak that is recognized early and subjected to vigorous control measures may extinguish.

To model the local outbreak, we used a branching process model, developed by, and in consultation with, Dr. James Lloyd-Smith (UCLA), a recognized world expert in stochastic epidemic modeling.[345] Branching process models are stochastic, where each case creates a number of new cases based on a probability distribution. In the model used in this report, the distribution is a negative binomial distribution with parameters R_0 (the average number of new cases each case generates) and k (which reflects the variation in infectiousness between individuals, where low values of k imply high variation

[345] J. O. Lloyd-Smith, S. J. Schreiber, P. E. Kopp & W. M. Getz. Superspreading and the effect of individual variation on disease emergence. *Nature* 438: 355-359 (2005)

and high values of k imply low variation). Low k is appropriate for MERS/SARS because most people create no secondary cases, whereas some create a very large number. Higher k is appropriate for flu because many people infect one or two others, some zero, some a large number. The range of values used for R_0 and k for wild type strains of influenza and coronaviruses is provided in the Supplemental Information. Branching process models capture one crucial feature of new outbreaks: that many new outbreaks extinguish at a small number of cases.

The branching process model we adapted considers various control measures and can account for partial immunity in the community (important for outbreaks of recently circulating influenza strains). Social distancing and isolation/quarantine are parameterized. Because our analysis is not to evaluate control measures but to compare the risk of various outbreaks, we explore a variety of plausible values for the parameters describing these measures. The parameter values that describe control measures are described in the Supplemental Information. Notably, our model tracks laboratory workers and community members separately so that we subjected each to different control measures.

In our analysis, an outbreak was considered to be out of control if either of the following conditions were met:

- The model calculated that, given the number of cases in the current generation, that the outbreak had less than a 5% chance of extinguishing at any point in the future, or
- That any generation included more than 1,000 infected individuals (which probably outstrips the ability of a locality to control), or
- The model includes 200 generations of infected individuals without extinguishing or reaching any other termination condition (suggestive of never getting under control).

In this Risk Assessment, 2.6 billion simulations were performed in our BPM to provide statistically sound data to explore the parameter set for wild type and GoF pathogens and a variety of outbreak control parameters.

Once an outbreak was considered out of control, it was considered to seed outbreaks globally. The illnesses and deaths due to an outbreak that extinguishes either due to stochastic forces or due to control measures were tallied as part of the consequences of the local outbreak.

6.2.7 Modeling the Global Consequences of a Human-Transmissible Outbreak

Once an outbreak was found to grow out of local control using the branching process model, we modeled the global consequences of a pandemic using the HHS-BARDA Interactive Influenza Model (IIM), which is used by the Centers for Disease Control and Prevention and HHS-BARDA to evaluate the effectiveness of medical countermeasure strategies to control influenza outbreaks.[346] IIM is a "Susceptible, Exposed, Infectious, Recovered" (SEIR)-based model, which is a compartmental epidemiological model which tracks the progression through various stages of a disease course of individuals in an outbreak. IIM considers the differences in vaccination and clinical visit rates of different age groups (children, adults, and the elderly), contact rates between these groups, and control measures, like mass vaccination, social distancing and antiviral treatment.

[346] For example, see Biggerstaff, M et al "Estimating the potential effects of a vaccine program against an emerging influenza pandemic--United States." Clin Inf Dis S1: S20-9 (2015).

IIM was developed using contract rates and demographic data for the US. To globalize the model, we collected demographic data for 12 regions of the world, divided by geography and income (with the rationale that high-income countries have distinct demographics and public health resources than other countries). We characterized each region by population, class size (used to scale school-based contact rates), household size (used to scale household contact rates), and age stratification (used to scale relative numbers of children and the elderly). The methodology for scaling contact rates is described in Appendix III Section 14.3.1 and the demographic data supporting the regionalization of the globe is provided in the Supplemental Information. Although this method captures some demographic differences between regions of the world, it does not capture cultural practices and socioeconomic factors (like underlying poor health) that could affect the outbreak. Also, public health measures, like social distancing, are assumed to be equally effective in all parts of the world (however, vaccine doses and antivirals are more limited).

If an outbreak escaped local control, we assumed that it would continue to seed infections in the US and a US-wide outbreak will continue to seed outbreaks abroad. For this reason, travel rates were unnecessary to obtain as eventually the disease would spread. Each region was seeded with 100 initial cases. Parameter values used in the IIM model are provided in the Supplemental Information.

To support the analysis in this Risk Assessment and adequately explore the parameter space, the IIM ran approximately 750,000 simulations.

6.2.8 Simplified Modeling of Bird-Transmissible Pathogens

One hypothetical consequence of a laboratory release of research with a strain of influenza that is transmissible only amongst avian species is that the strain could establish itself in wild bird populations (by infection via an aerosol, contaminated worker, or contaminated waste leaving the laboratory), causing sporadic human disease over a dispersed geographic area, similar to the natural H5N1 strain today. For this eventuality to occur following loss-of-containment and subsequent release, a series of events must occur: the virus must reach an environment where infection of a wild bird can occur; it must infect a wild bird; the virus must spread and migrate with a population of birds; these infected wild birds must then spread the virus to a domestic bird population; this virus must then spread from the domestic birds to humans; and finally, the virus need be capable of causing disease in a human host. Note that a virus that spreads between humans is presumed to spread between humans efficiently and any incidental transmission from birds will not significantly affect the kinetics of the outbreak; hence, this section does not consider human transmissible viruses. This presumption is supported by the opinion of several of the interviewed experts, who believed that a Gain of Function influenza virus, including the H5N1 strains adapted to transmit between ferrets by the airborne route, could be adapted to spread efficiently among humans or among birds, but not between them due to differences in viral receptors in these animals. This belief agrees with the historical evidence, as we have yet to identify either a natural human influenza that spreads easily among birds or a natural avian adapted virus with sustained mammalian transmissibility.

6.2.8.1 Unpredictability of the Consequences of Novel Avian-Influenza Strains

Determining the probability and consequences of each of the events necessary for an avian virus to infect humans is very difficult primarily due to missing data. For example, one reference reviewed 4,763 literature sources of human to animal transmission of any disease, and found no documented examples of direct human to animal transmission of influenza.[347] Similarly, despite detection of influenza in natural water sources and measurements of the persistence of influenza in water suggesting that "cloacal

[347] Messenger AM *et al* (2014) Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. *PloS one* 9: e89055

drinking" by waterfowl of contaminated water may be a source of infection, no identified source listed an ID_{50} for such a process.[348,349,350] However, of the sources of uncertainty, estimating the consequences to humans of a flu circulating in wild birds remains the largest due to uncertainties in the biology of the virus and the role of human-avian interaction in its epidemiology.

Despite intense research efforts spanning decades, predicting the transmissibility and pathogenicity of a new or novel avian strain in humans or other mammalian hosts remains challenging. Part of this difficulty stems from the diverse range of symptoms and effects seemingly similar strains cause, combined with the apparent uncorrelated symptom severity between birds and mammals. Shown in the Supplemental Information are data for eleven recent avian influenza outbreaks, eight of which have caused human cases. Comparing the outbreaks reveals the unpredictability of human effects. For example, despite the virus that caused the 2015 H5N2 outbreak containing a hemagglutinin (HA) in the same clade as one known to cause fatal human H5N1 infections, the H5N2 outbreak has of yet caused no known human cases of infection.[351,352] This difference could be behavioral (due to enhanced biosafety practices in the poultry industry in the USA) or may be due to differences in biology of the strains. The H7N7 outbreak of 2003 caused only one human fatality, and most symptoms were restricted to conjunctivitis even though the strain appeared highly infectious to humans, with 250/500 of potentially exposed humans tested showing evidence of seroconversion.[353] In comparison, the ongoing H7N9 outbreak causes minor to no signs in either wild birds or poultry, but causes severe respiratory disease in humans in the relatively few human cases it has caused.[354]

The distribution of an outbreak is as unpredictable as its transmissibility and pathogenicity. The majority of poultry outbreaks of influenza remain constrained to one or a few flocks, with a few spreading much further. The current outbreak of H5N1 began in December 2003 with the first reported human cases in Vietnam and spread rapidly.[355] By April 2004 it had spread to Thailand, Korea, Japan, Indonesia and Hong Kong and by November 2004 to mainland China. By February 2006 it had become intercontinental, spreading to Europe as well as Africa where it remains endemic to Egypt. The timing and location of spread appeared to correlate with bird migratory patterns, hinting at wild bird-mediated spread.[356,357] In contrast, H7N9 began in the same global region, and appeared to initially spread more quickly, yet despite beginning in the same region and presumably being subject to the same cultural and geographic factors, it has only spread through a geographically contiguous area and not spread internationally, confounding determination of whether the spread is primarily wild bird or human mediated.[358] Meanwhile, the North

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348 Deboosere N *et al* (2011) Development and validation of a concentration method for the detection of influenza a viruses from large volumes of surface water. *Applied and environmental microbiology* 77: 3802-3808

349 Stallknecht DE *et al* (1990) Persistence of avian influenza viruses in water. *Avian diseases* 34: 406-411

350 Alexander DJ (2007) An overview of the epidemiology of avian influenza. *Vaccine* 25: 5637-5644

351 Ip HS *et al* (2015) Novel Eurasian highly pathogenic avian influenza A H5 viruses in wild birds, Washington, USA, 2014. *Emerging infectious diseases* 21: 886-890

352 de Vries E *et al* ibid.Rapid Emergence of Highly Pathogenic Avian Influenza Subtypes from a Subtype H5N1 Hemagglutinin Variant. 842-846

353 Fouchier RA *et al* (2004) Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proceedings of the National Academy of Sciences of the United States of America* 101: 1356-1361

354 World Health Organization, "Overview of the emergence and characteristics of the avian influenza A(H7N9) virus", Report issued May 31, 2013.

355 Yee KS *et al* (2009) Epidemiology of H5N1 avian influenza. *Comparative immunology, microbiology and infectious diseases* 32: 325-340

356 Liang L *et al* (2010) Combining spatial-temporal and phylogenetic analysis approaches for improved understanding on global H5N1 transmission. *PloS one* 5: e13575

357 Gilbert M *et al* (2006) Anatidae migration in the western Palearctic and spread of highly pathogenic avian influenza H5N1 virus. *Emerging infectious diseases* 12: 1650-1656

358 Bui C *et al* (2015) A Systematic Review of the Comparative Epidemiology of Avian and Human Influenza A H5N1 and H7N9 - Lessons and Unanswered Questions. *Transboundary and emerging diseases*

American H5N2 outbreak began in the Pacific Northwest and quickly jumped two migratory flyways to the Midwest; the mechanism for this rapid eastward spread has not yet been identified.

The lack of a solid scientific evidence basis for predictive epidemiology in avian influenza viruses implies that any serious quantitative analysis would be unfounded. For this reason, we took a simplistic approach to modeling outbreaks of influenza viruses that spread between birds only.

6.2.8.2 Spread of Escaped Laboratory Virus to Wild or Domestic Birds

First, avian-influenza strains are modeled in the Fault Tree Analysis like any other strains. We have enough data to predict the chance of infection of a human or a bird when exposed to a source of pathogen. We can therefore quantitatively predict if humans or animals are infected within the laboratory (due to a variety of incidents) or outside the laboratory (due to aerosols or transfer of contamination from a worker to poultry). Should a bird be infected outside the laboratory or an infected bird escape from the laboratory (in the earthquake and biosecurity scenarios), we presume that an avian influenza outbreak occurs and has consequences similar to the recent outbreaks. That is, we presume that between 0 and 1,000 human infections occur and that the case fatality rate is between 0 and 50%. Given the lack of data, our model presumes an equal probability of any result in this range. Because we are not estimating economic consequences or risks to animal health, this approach is sufficient to characterize the risk of this agent to humans given the paucity of data available.

Recall that if the pathogen is transmissible between people (regardless of if the strain is a natural one or if it is a modified avian-influenza strain), we modeled the outbreak assuming that all human health risk is dominated by human-to-human contact.

6.2.9 Estimating Risk of Experiments Involving GoF Pathogens

Each modeling component is used to predict a single aspect of risk:

1. The Fault Tree Analysis is used to determine how pathogen characteristics, containment features, experimental manipulations and the laboratory environment contributes the *probability* of escape, and the number of cases that would initiate an outbreak (a component of consequence),

2. The branching process model estimates the *probability* that a local outbreak would grow and seed a global pandemic. If the outbreak extinguishes due to stochastic factors or due to an effective public health response, the *consequences* from the local outbreak are tallied, and

3. The HHS-BARDA Interactive Influenza Model is used to predict the global *consequences* of a pandemic.

By linking the outputs of the modeling component, we can state how much any pathogen, research feature, or environment drives risk. For example, we can ask the specific question of how much does fatality risk change if one increased the transmissibility of H5N1 influenza in humans to half of that of seasonal influenza. We would explore how the probability and consequences of all laboratory accidents depends on this change, and how the probability of a local outbreak escaping control depends on this change and how the consequences of a global outbreak depend on this change. Comparing the three modeling components together provides an overall estimate of the change of risk.

In fact, for each pathogen phenotype and condition under which GoF research could be performed, we determined how varying the phenotype or research condition within scientifically defensible limits

influences risk. To undertake this sensitivity analysis, we determined how risk changes if a parameter value is held to a series of specific values in a Monte Carlo analysis in which other parameter values are allowed to be selected at random. This analysis determined if risk increases or decreases as any specific parameter value changes across the range of possible values for all other parameters.

Each parameter that is found to significantly influence risk (either positively or negatively) compared to a baseline that assumes work with unmodified pathogens was further explored to understand the reason behind this relationship. In this way, we determined if a parameter value must be set to an unreasonable value in order to significantly drive risk or if risk can be increased at parameter values that could be easily expected. Moreover, by analysis of the results, we determined if risk is driven only when a combination of parameter values occurs (for example, risk increases significantly only if the pathogenicity *and* transmissibility of an agent is increased, or only if transmissibility is increased and the work is performed without worker health monitoring). Together these results will help identify the GoF activities and conditions that could significantly increase risk of an outbreak compared to work with wild type pathogens.

The branching process model and the IIM are computationally intensive and so a Monte Carlo analysis could not be done to explore the entire parameter space. Instead, a variety of discrete parameter values were rationally chosen to defining the epidemiology (e.g., R_0 and latent period) of the viruses and the efficacy of control measures to contain the outbreak. For each of these parameters, a range of values believed to cover a significant fraction of the possible parameter values were used, and results were obtained for each unique combination of every parameter varied. Figure 6.12 illustrates the variation in simulation results for these parameters for seasonal flu global outbreaks, where each marker represents a result for a unique combination of parameters.

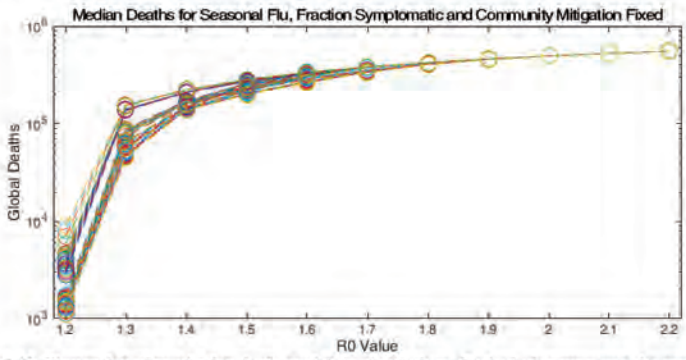

Figure 6.12. Illustration of results for single models in simulations. Each circle represents the results from a single model run with a single set of parameters. Lines connect models that differ only in the R0 value used in the simulation. To reduce the number of lines for visual clarity, some parameters were fixed: shown are models using the median values of antiviral efficacy on mortality, case fatality rate, and fraction of cases symptomatic, as well as no community mitigation; all other parameters ranged across their values appropriate for seasonal influenza.

Elsewhere in this report, when showing results for these simulations, the median number of deaths across all values of the varied parameters is shown by the marker, and the 10th and 90th percentile value of deaths across the same parameters are shown by vertical lines extending outward from those markers (the "error lines"). These vertical lines do not represent statistical (aleatoric) uncertainty in the underlying

simulations; instead they represent uncertainty as to the properties of the virus, outbreak and public health capacities (epistemic uncertainty). If a real-world outbreak were to occur with a defined set of parameters matching those simulated by one of the models, the results would, with high confidence, match those of that particular model. In addition, while these vertical lines plotted cover 80% of the resultant number of deaths for the model parameters simulated, they should not be understood as a typical 80% confidence interval. Because no probability distribution was assigned to the underlying varying parameters, the vertical lines represent *the middle 80% of the outputs of the simulations*, and not *the range of the 80% most likely outbreaks*. This approach suggests that a real outbreak would follow the overall shape of the median line presented (and not simply move randomly inside the range presented), but may be higher or lower (up to the bounds of the "error bars") if certain properties of the virus or the control measures caused the outbreak to spread more aggressively (or less aggressively) than the median set of parameters.

6.3 Practices in GoF Laboratories That Reduce Risk but Are Not Included in Our Study

To collect data to inform the modeling approach, interviews were conducted with laboratorians, biosafety officials, and public health officials. These interviews uncovered several measures that certainly reduce the risk posed by containment research but in ways that could not be included in a quantitative study that models human behavior abstractly. This section describes some of these practices that speak well of the culture of biosafety that exists in these containment laboratories but could not be captured by our models.

A thorough examination of current practices in influenza and coronavirus biosafety level 3 (BSL-3) was conducted through site visits and interviews with researchers, public health officials, and institutional representatives. Best practices in biosafety and biosecurity pertaining to Gain of Function research were identified that exceed recommendations or requirements from various bodies, including the Occupational Safety and Health Administration (OSHA), select agent regulations, recommendations of the Federal Experts Security Advisory Panel (FESAP), and Institutional Animal Care and Use Committees (IACUC). Practices either unique to specific institutions or commonly found across institutions are highlighted and were found to be especially beneficial/optimal/useful in training, exercises and drills, laboratory practices, health precautions, physical security, and institutional culture.

6.3.1 Training

Laboratory directors and personnel at various BSL-3 laboratories shared their protocols for training new researchers. From this, several best practices are highlighted. One observed prerequisite to BSL-3 work is demonstrating competency in BSL-2 work. Additionally, across all institutions, extensive BSL-3 training was observed, involving both written examinations and supervision of hands-on laboratory skills. One institution described a tiered training structure, in which the first tier covered basic laboratory operations, emergency situations, and general laboratory safety. The second tier covered more specific training for laboratory safety when performing cell culture work. The third tier covered procedures and precautions for animal work. Each tier was associated with a training checklist, which a trainer would use to assess the trainee. Another institution required the trainee to shadow the trainer in the BSL-3 laboratory and perform laboratory procedures under mentored supervision before conducting independent work. Best practices for hands-on training involve dedicated one-on-one instruction and active roleplay for scenarios such as an animal bite or a biohazardous spill.

Training and education can be codified into standard operating procedures (SOPs) covering experimental protocols, biohazardous spills, working with animals, potential exposure to infectious material, and biosecurity threats. These SOPs can be made easily accessible within the BSL-3 containment lab, should the need arise. Demonstrating knowledge of all SOPs can be required as part of BSL-3 training. Additional training in biosecurity is also recommended, covering topics such as cybersecurity, identifying

abnormal or suspicious behavior, identifying insider and outsider threats, and how to deal with strangers requesting lab access. Institutions remarked on the need for constant reminders and renewal of training to counter complacency. Commendably, some institutions were particularly thorough about BSL-3 training requirements. Visiting researchers were required to repeat BSL-3 training, even if they had prior experience either elsewhere or at that same institution. Training is not only limited to issues of biosafety and biosecurity. One institution provided communications training for researchers on how to discuss Gain of Function work in public settings. Finally, institutions can offer select agent training to first responders to inform what agents may be present during an incident and what to do in case of a large-scale spill or a fire.

6.3.2 Exercises and Drills

Hands-on training can extend beyond laboratory protocols to tabletop exercises and drills within and outside the laboratory setting. Institutions described several exercises and drills, such as responding to a researcher having a medical emergency in the BSL-3 containment lab, responding to a potential exposure in the laboratory, and responding to a natural disaster. Another research facility discussed methods for testing their security infrastructure, such as leaving a door open or holding up signs to security cameras to test for prompt response. On a wider scale, the research institution can conduct exercises and drills in conjunction with first responders, environmental health and safety (EHS), and local hospitals for better preparation against a potential exposure. Examples of such exercises are: a researcher following SOPs for exposure to a pathogen, a researcher not following SOPs for exposure and showing up at a hospital emergency room, and response to a bomb threat. Conducting these drills also strengthens cross-institutional relationships, which can better inform future preparedness and response protocols. One institution asked local first responders to perform walkthroughs of the research facility to learn how to gain access and what to do during an emergency. For instance, the fire department was instructed to contain but not extinguish a fire in the BSL-3 containment lab, allowing it to burn within those boundaries. Notably, one institution remarked that whenever a researcher would display influenza-like illness, this essentially became an exercise in practicing SOPs for a potential exposure. Finally, a best practice that formalizes these relationships is to establish an Emergency Operations Center (EOCs) under the parent institution or university to better coordinate emergency responders, EHS, local public health, and the research facility. EOCs can run campus-wide drills to scenarios such as bomb threats, natural disasters, and active shooters to prepare a coordinated response effort from multiple agencies.

6.3.3 Laboratory Practices

CDC select agent regulations dictate several requirements for day-to-day laboratory operations, including a regular inventory of pathogen stocks and inspections of laboratory equipment and the BSL-3 facility. Several institutions have demonstrated particularly useful laboratory practices that may surpass regulatory requirements or otherwise represent optimal biosafety and biosecurity measures in access control, inventory, animal work, facility maintenance, and communications. Furthermore, select agent requirements represent best practices for non-select agent labs that work with, for instance, seasonal influenza viruses.

Several best practices in access control are highlighted here. One non-select agent status laboratory was observed to keep its freezer containing pathogen stocks under lock and key and to perform frequent inventory checks. Another practice was to grant access to select agent freezers only to a small number of staff out of the many more who were approved for BSL-3 work. This can prevent researchers from performing unauthorized experiments, as it requires explicit permission to access the pathogen stocks. Another institution required researchers to obtain permission to access anesthetic drugs for anesthetizing

animals for *in vivo* work. More broadly speaking, it would be a best practice to control access to reagents necessary for risky experimental protocols.

Maintaining an updated inventory is a requirement for laboratories working with select agents. However, one institution was noted to count inventory more frequently compared to peer institutions (monthly instead of quarterly). Alternatively, another facility performed random inventory checks. One institution randomly sampled 10% of its boxes to reconcile its contents with the inventory log. If discrepancies were noticed, a 100% inventory check was performed, and the CDC was notified. Another select agent requirement is to limit how long experimental samples may be kept. The best practice observed for this requirement was to keep experimental samples up to 30 days, after which they were either discarded or added to the permanent inventory. There are several additional best practices associated with counting inventory. One institution required two people present to count inventory. One researcher was "permanent" and was always present at every inventory check. The other researcher was "rotating" to witness the inventory count and ensure that inventory was not simply memorized as a complacent way of counting. Another institution assigned one employee to keep track of all changes to inventory; this employee was responsible for conducting counts and was to be notified if a sample were to be taken from stocks.

Additional practices were noted that improve the safeguarding of inventory. Witnesses can be required for any changes to inventory, including taking agents from pathogen stock, destroying old samples, and adding samples. Stocks not used for at least one year can be archived in boxes sealed with security tape.

Researchers highlighted several best practices when working with animals in the course of pathogenic research. One is to limit researchers' and animal caretakers' contact with laboratory animals, a USDA regulation though not a CDC regulation. Animals can be observed prior to the conduct of experiments to determine whether they are prone to abnormal or aggressive behaviors, which may make them more likely to inflict bites or scratches on their handlers. These behaviors will be noted for experimenters so they can take appropriate precautions when working with those animals. Furthermore, animals can be completely or partially anesthetized before experimental procedures to prevent bites or scratches. One research group noted that genetically mixed mice were more prone to aggressive behaviors and thus partially anesthetized all mixed mice as a precaution. A daily check, including weekends and holidays, of animals and other laboratory equipment can be conducted. In order to record which employees were trained to perform animal experiments, animal husbandry, and respiratory testing, one facility kept an animal handling training sheet. Finally, a paper trail for each laboratory animal can be maintained, which details its history of procedures, tests, and bodyweight measurements, as well as the dosage and strain of the experimentally induced infection.

Briefly, some best practices were noted with regards to maintaining the facility and its equipment. Frequent inspections of the shower and facilities can ensure that containment safeguards and decontamination procedures remain optimal. Additionally, several facilities performed annual shutdowns for several weeks in order to perform a comprehensive surface and gas decontamination and to perform preventive maintenance.

There were several practices observed that sought to optimize researcher-to-researcher communications or to utilize a partner system to limit mistakes or malicious behavior. A radio system can be used to communicate between BSL-3 researchers and outside staff. One institution mandates that any potential exposure, no matter how minor and even if it does not breach PPE or skin, should be reported over the radio. This allows an outside employee to be aware of the situation, and furthermore the employee can guide the BSL-3 researcher on next steps, preventing a possibly stressed researcher from making rash decisions. Another simple tool is to place a whiteboard outside the BSL-3 containment lab that displays which researchers are working in which suites, and which pathogens are in each suite. One best practice

that was especially notable was notification of weekend or after-hours work. Researchers seeking to conduct work off-hours can be asked to notify a coworker by phone of time of entry, expected duration, and time of exit. One institution employed an on-call cell phone, which is always kept on and is assigned to an employee by rotation. Messaging this phone is required for after-hours work in the laboratory. Finally, several institutions require BSL-3 laboratory staff to wear emergency "man-down" pendants, which can used in the event of an emergency to alert first responders and research supervisors.

With regards to a partner or two-person system, when interviewing different research institutions, different opinions emerged on its utility. Many institutions required the partner system when performing experiments requiring animals or sharps. Some institutions used the partner system liberally, requiring witnesses to validate changes to inventory (as mentioned above) or proper execution of inactivation protocols (inactivating an agent to transfer from BSL-3 to BSL-2). However, institutions differed in their opinions about the partner system when performing more routine experiments. One institution encouraged the partner system whenever possible. However, researchers at another remarked that the risk of accidental exposure was higher with two people, and that the two-person system provided little utility. It is important to point out that the utility of the two-person system has historically been contentious, and that no applied research has been done to assess the benefit of such a measure.

Lastly, some additional best practices for day-to-day laboratory conduct are to limit a researcher's hours in a BSL-3 lab to three to four hours daily and to designate one employee to receive and sign off on shipped biological materials. These can limit the chance of exposure and ensure an extra degree of security, respectively.

6.3.4 Health Precautions

Several best practices were identified that better reduce the chances of severe illness following a laboratory exposure. These can be categorized as conditions of employment, post-exposure SOPs, and partnerships with local and state public health departments and with local hospitals.

Some institutions were observed to require employees who worked in BSL-3 laboratories to abide by certain rules. One commonly observed best practice was the requirement of the seasonal influenza vaccination as a precaution against laboratory-acquired influenza infection. Another was to medically clear new employees, which would (1) discover any underlying medical issues that may exclude a researcher from working with select agents and (2) obtain a baseline serum sample prior to starting lab work, in order to test for seroconversion in the event of potential exposure. Lastly, one institution obtained signed statements from its employees agreeing to self-quarantine, self-report body temperature, permit home visits by a nurse, and submit samples for diagnostic testing, in the event of a potential exposure.

One best practice for post-exposure SOPs is to include extra precautions following a potential exposure. For instance, one institution isolates the exposed researcher and administers an N95 mask without an exhalation valve while awaiting emergency medical response, even though the pathogen would not be expected to replicate within those few hours following exposure.

Partnerships between the research institution, local and state public health, and local hospitals can be established prior to an exposure incident to expedite the medical response. Researchers can carry cards describing their occupation and what agents they work with, which should be shown at the emergency department to facilitate proper treatment. Medical emergency protocols for laboratory pathogens used in the neighboring research institute can be shared with the local emergency department, and occupational health concerns for working with these pathogens can inform hospital protocols for safety and security. In

fact, this can be further codified into a memorandum of understanding (MOU) with the local hospital. It was noted that if the institution is a university, hospital physicians can often be affiliated with the university's medical school, which facilitates a culture of cooperation between the hospital and research staff. The contributions of institutional culture to best practices in biosafety and biosecurity are explored in a later section. Finally, one institution has shared samples and genomic sequences with the state public health department to verify that their diagnostic tests detect the virus strains commonly used in the laboratory, in the event of a potential laboratory-acquired infection.

For employees leaving the university, they must terminate access two weeks prior to leaving and go through an exit physical before they leave, to ensure they're not sick (two weeks based on incubation period of SARS/MERS – ten days). In some laboratories, everyone must check into lab daily. If someone doesn't show up, the lab is responsible for tracking them down. Lab will notify EHS if they are unaware of someone's whereabouts, and EHS will reach out to the university hospital ER to let them know to watch out for that person to show up.

6.3.5 Institutional Culture

Several researchers cited their institutional culture as a powerful factor in promoting safety and security in the laboratory. Institutional culture can dictate workplace satisfaction, willingness to report incidents, awareness, and workforce turnover, all of which can directly or indirectly influence the levels of biosafety and biosecurity. Several institutions noted the importance of developing a non-punitive culture that encouraged over-reporting, especially of "gray-area" incidents such as a minor spill without breach of PPE or skin. Also widely practiced was a culture of carefulness and vigilance, bolstered by consistent reminders to practice good safety and security measures to prevent complacency. One institution remarked that the principal investigator sets the example by obtaining all biosafety and biosecurity training. Many institutions cited their small work environment as conducive to maintaining vigilant security, since all of the staff knew each other. One supervisor commented about developing an intuition for the happiness levels of all staff members, which can reduce the risk of an insider threat. Institutions can additionally offer assistance programs for employees to cope with hardships or obtain counseling. Establishing an environment that promotes staff retention is also a best practice. This builds relationships between laboratory staff and is a strong security measure in limiting the number of new employees. Additionally, one institution does not allow undergraduate students to work in the BSL-3 lab, due to concerns with turnover and with the length of time needed for BSL-3 training.

Strong support from the parent institution for the Gain of Function research program can also promote a positive working culture. One research group noted that, in the face of controversy, the parent institution remained strongly supportive of the research program, which encourages the laboratory staff to be diligent about reporting incidents and maintaining a safe and secure working environment.

Finally, as mentioned above, strong relationships between the research institute and local hospitals, first responders, regional FBI offices, EHS, and local and state public health departments contribute to a positive institutional culture that lends itself to better preparation for and response to laboratory incidents.

6.3.6 Additional Institutional Policies

Finally, additional best practices were observed in various institutions that do not fall under the above general categories. Several institutions required principal investigators to register their research with EHS, documenting a notice of intent (listing the agent of study, purpose of the study, and dual use research questions), a risk assessment, and training requirements. As part of this registration, EHS can perform an annual inspection of the facilities to verify the proper safety and security measures. Institutions have also

employed campus-wide behavioral risk assessments to monitor for behaviors or emails of concern. Finally, institutions can share their own practices with other research facilities, improving each other's security and safety procedures.

6.4 Probability of Laboratory Acquired Infections

6.4.1 The Selection of Incidents to Include in the RBA

In this study, we analyzed ten previous laboratory accident risk assessments and three compilations of accident/incident reports to identify the accident or incident scenarios that would be quantitatively evaluated in our study. Any scenario that was high risk (either due to their frequency or consequence) or used as the "maximum reasonably foreseeable events" in *at least* one source was included for quantitative analysis, except when:

- General accident types that are explored in more detail by another accident type (e.g., "waste stream" would be discarded in favor of the high risk "leaking pipe" scenario),
- Any incident with an unknown cause because these are not quantifiable (e.g., "contamination outside laboratory with unknown cause"—note that these are likely captured by other event types), and/or
- Accidents specific to containment research on large animals

Other scenarios were considered but not included in a quantitative analysis because they are rarer than events that would have a larger consequence. Beyond these events, we included additional scenarios to capture risks that may inhere in GoF research specifically or were recently in the news, specifically:

- Floods, due to the flooding of hospitals and laboratories that occurred during Hurricane Sandy, and
- Animal bites because of extensive work with ferrets, which tend to bite more often than mice or guinea pigs

In many cases, the "incidents" identified in other reports aren't incidents in themselves but risk factors that influence the risk of other incidents. That is, if the HVAC system fails, this failure has a consequence only if animals are actively exhaling pathogen into the ambient air or there is a spill or splash. For these events, their probability of occurrence was included in ALL other relevant accident fault trees. In total, 16 incidents were investigated in detail to form the basis of our quantitative analysis.

Table 6.1. Rationale for Scenarios Included in the Risk Benefit Assessment.	
Scenario	**Rationale for Inclusion**
Splash incident	Recognized as high risk in the NBAF
Spill incident	Recognized as high risk in NBAF
Failure to keep containment in place	Recognized as high consequence in NEIDL. Included as a factor in other incidents
Solid waste incident	Recognized as high risk in NBAF
HVAC failure	HVAC failure was noted in reports and could be of potential high consequence. Included as a factor in other incidents
Equipment-- failure of containment feature	Common in incident reports. Included as a factor in other incidents
Equipment--power loss at facility	NEIDL estimates high risk and actual examples in reports
Improper inactivation of pathogen	Many examples in reports, human error (with equipment failure, human failure to check inactivation (e.g., recent anthrax at DoD)). Investigated separately but also is part of other incidents investigated (waste streams and splashes).
Transference--glove to skin due to improper removal	High risk in NBAF. Included as a factor in other incidents
Shipping accident	Considered exceptionally high risk in NBAF; examples in reports of accidents (though of no consequence)
Animal--escape from containment	Recognized as high risk in the NEIDL while other RAs state low risk; some examples in literature of animals escaping or otherwise disappearing
Improper inactivation of liquid waste	Recognized as high risk in NEIDL
Centrifuge release	Canonical high risk scenario in almost every RA
Natural Disaster--earthquake	Most catastrophic scenario for NBAF and NEIDL
PPE Failure	Several examples in literature leading to actual laboratory acquired infection
Protocol failure--use of wrong containment	Several examples in literature leading to actual LAI. Included as a factor in other incidents
Puncture/sharp object injury	Puncture is the most common cause of reportable lab accidents. Because the GoF pathogens are probably not infectious via injection, this incident is considered to lead to a breach in the gloves that creates a contamination on the hands (leading to possible later inoculation of the worker or a contact).
Waste-liquid waste leak/pipe burst	High risk in NEIDL
Animal--bite/scratch	Although low risk in NEIDL, may be higher frequency in ferrets
Exhaled pathogen escapes laboratory (animal respiration)	Due to contagious nature of some GoF pathogens, this scenarios deserves quantitative evaluation
Natural Disaster--flood	Low risk but recent examples present - Galveston and New York
Scenarios listed in blue are failure modes that could exacerbate the risk of a loss of containment and are included in other events. Scenarios listed in green were included by name in this assessment.	

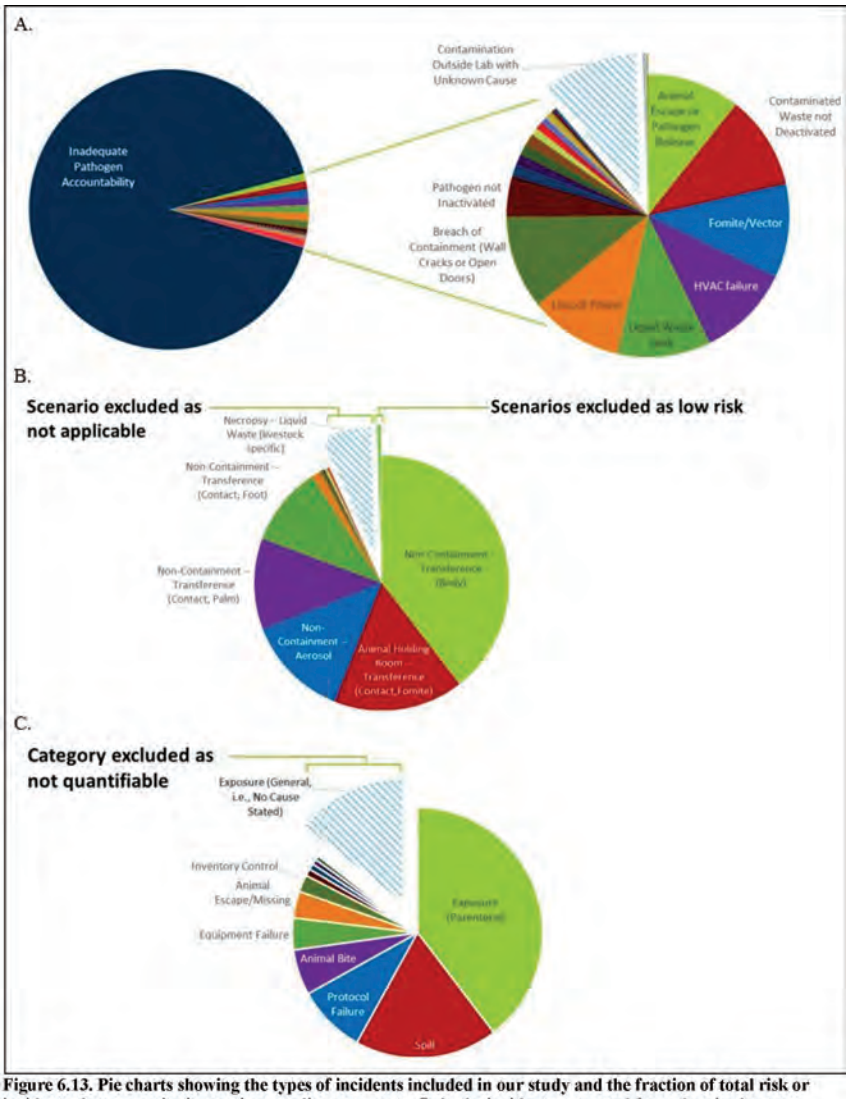

Figure 6.13. Pie charts showing the types of incidents included in our study and the fraction of total risk or incidents they comprise in previous studies or reports. Only the incidents extracted from the pie chart are excluded from further analysis in this RBA. A. The NEIDL, B. The NBAF, C. past reports.

Note that these events include the low probability, high consequence incidents; the high probability, high consequence incidents; and the high probability, low consequence incidents from other reports, along with the "maximum reasonably foreseeable events" incidents. Excluded from this point on in the assessment are only the events that are rare and inconsequential, or not foreseeable (for instance, have a probability of less than the age of the earth). Note, any event that is reasonably foreseeable (even if extremely unlikely) and of high consequence was captured in our assessment. These 16 incidents capture the vast majority of the risk from previous assessments: more than 99% of the risk from the NIEDL study, about 90% of the risk from the NBAF study (much of the rest relates to work with large animals only), and 80% of incident reports (the rest are not quantifiable due to unknown cause) (Figure 6.13 above).

All incidents included in our study were studied to reconstruct the pathways leading to the loss of containment event. After study, some incidents were excluded from quantitative analysis because no plausible scenario that leads to a loss of containment could be identified to model. Other incidents were quantitatively investigated but excluded from the fault tree analysis because all pathways identified lead to vanishingly unlikely and/or small releases. See Appendix III for details.

6.4.2 Identification of Locations at Risk of Earthquakes and Floods

To quantitatively assess the risk of earthquakes and floods at GoF laboratories, we found the GPS coordinates of 36 containment laboratories, including labs that were formerly conducting GoF research and several additional BSL-4 and BSL-3 facilities that are currently operational or under construction. The flood risk at each location was assessed using information from the Federal Emergency Management Agency.[359] The earthquake risk was assessed using information from the US Geological Survey.[360] Of these 36 locations we identified the locations of greatest risk of flooding and earthquake and assessed the risk of a loss of containment event at that facility due to a natural disaster. If the risk was significant, we would have assessed the risk from these natural disasters at other sites with slightly lesser risk. However, we found that the risk of natural disasters was minor compared to accidents, so this analysis was not performed.

6.4.3 Irreducible Uncertainty Prevents an Accurate Prediction of Absolute Risk

Humans are an integral component of every laboratory, however, humans are prone to making mistakes due to carelessness, haste, tiredness or unfamiliarity with validated procedures. In most complex systems, the physical systems (fans, valves, filters, alarms) are demonstrated to fail much less often than the humans operating the systems and interpreting the alarms these systems make when an error occurs. The only human reliability data found directly related to work in a containment laboratory are studies of decontamination (when removing gloves or washing hands). Much of the data on human reliability comes from the transportation, chemical and nuclear sectors and this study had to analogize to interpret human error rates to laboratory situations. Because of the absence of data, in this risk assessment some conservative assumptions were made that prevent the accurate estimation of absolute risk. None of these assumptions affect the relative risk of an accident with a modified pathogen compared to a wild type and so the comparative risk assessment still holds.

Conservative assumptions made include:

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[359] FEMA, National Flood Hazard Layer Map (Official), accessed in June, 2015 at http://fema.maps.arcgis.com/home/webmap/viewer.html?webmap=cbc088e7c8704464aa0fc34eb99e7f30.

[360] USGS, US Seismic Design Maps, accessed in June, 2015 at http://earthquake.usgs.gov/designmaps/us/application.php

- When a skill error (a slip) happens with a sharp object (scissors, typically) during a necropsy, the assumption is that the slip results in a cut through the worker's glove(s). There are no data on the relative number of errors during necropsy that result in damage to the specimen, dropping of the instrument or any other inconsequential failure compared to breaches in the gloves, therefore this assumption was made to conservatively maximize risk.

- When a splash happens when working with pathogen (for example a contaminated pipette tip skipping over the top of a well), the splash is assumed to land on the worker's hands and not the hood or any part of the clothes or body unlikely to contact the worker's face or others outside the laboratory. There are no data on distribution of drops from laboratory accidents on this scale so the assumption of contamination on the gloves was made to conservatively maximize risk.

- When a worker contaminates their hands by any pathway, the contamination is assumed to be on the fingertips because this part of the hand is mostly likely to contact a contaminated surface. This is a conservative assumption because the fingertips are the only part of the hand to permit a self-inoculation (in the eye or nose) to maximize risk.

- When gloves fail, they are assumed to fail on the fingertips because these parts of gloves are the most prone to failure. Note that this assumption forces the point of contamination and glove failure to be coincident, which maximizes risk.

- When an accident the worker directly caused leads to the generation of an aerosol (like the spill of a viral stock), the assumption is made that the worker inhales all of the aerosolized pathogen because they are nearest to the most concentrated part of the spill (assuming that the aerosol reaches equilibrium in the room does not account for the fact that the worker was relatively close to the source).

6.4.4 Relative Probability of Laboratory Acquired Infections

The approach used in this risk assessment predicts that a variety of accidents, when combined with human or equipment failures, lead to laboratory acquired infections from work with the GoF pathogens. For seasonal influenza, most laboratory acquired infections are the result of aerosols accidentally generated by spills or centrifuge accidents, while a minority are caused by contamination of the hands during necropsy, cell culture, or via an animal bite. For pandemic influenza, because of the additional respiratory protection used under BSL-3 conditions, events that contaminate the hands cause slightly less than half of the laboratory acquired infections, while the rest are caused by aerosols. In avian influenza laboratories, the vast majority of infections are those of wild birds contaminated by the accidental discharge of incompletely decontaminated solid waste. Less than 10% of the accidental infections caused in avian influenza laboratories are in the human workers. For the coronaviruses, even though additional respiratory protection is worn under BSL-3 conditions, most infections are caused by aerosol exposure because other routes are unlikely to cause an infection. Although this analysis produces a robust estimate of relative risk in a variety of informative ways, the data used are insufficient to predict absolute risk. A separate method is used to support a rough estimate of absolute risk in Section 6.8.

In sum, the analysis of these release pathways enables the estimation of the relative risk of working with the GoF pathogens and how the change of any phenotype would alter this risk. Table 6.2 shows the relative probability (compared to work with seasonal influenza) of a laboratory acquired infection (that produces some hazard of a causing a local outbreak) when working with the various pathogens considered in this study. Our analysis considers that vaccination of laboratory workers could reduce the chance of a laboratory infection and that antivirals could be given prophylactically if a high risk exposure event

occurs. Moreover, health monitoring and isolation protocols would greatly reduce the chance that a worker mingles with the general population, causing secondary cases and sparking an outbreak. In this section, these factors are always considered when examining the pathways that lead to a laboratory acquired infection, because if the infected worker poses no hazard to the population, the consequences of the accident end with that person.

Table 6.2. Relative Probability of a Laboratory Acquired Infection for the Various Pathogens Considered in This Study as Compared to Work with Seasonal Influenza

Pathogen	Biosafety Level	Relative Probability of an LAI*
Seasonal influenza virus	BSL-2	1 (defined)
Pandemic influenza virus	BSL-3	0.10 (0.07-0.15)
Avian influenza virus	BSL-3	0.43 (0.21-0.90) (mostly of birds)
SARS-CoV	BSL-3	0.03 (0.02-0.04)
MERS-CoV	BSL-3	0.01 (0.006-0.02)

These data are generated by comparing the sums of the frequency of infection from all loss of containment pathways for each pathogen. In this case, we use the term laboratory acquired infection to include an infection of wild birds to capture the comparative risk of working with avian influenza viruses. The numbers in the parenthesis are the results from the p5 and p95 outputs of the Monte Carlo analysis.

The irreducible uncertainty in the pathways that lead from laboratory incidents to infections of wild birds with avian influenza is evident in these results. As will be described below, if infected material leaves the laboratory, it is assumed that wild birds will access it at the dump because there is no way to estimate what percent of bags are accessed by birds in a dump. The estimate here is therefore conservative but, even with the uncertainty provided, suggests that the probability of a wild bird becoming infected in a laboratory accident with avian influenza is roughly equivalent (within an order of magnitude) to the probability a person will be infected by a laboratory accident involving seasonal influenza. In contrast, the risk of an accident leading to an infection with any other pathogen is roughly one (for pandemic influenza) or two orders of magnitude less (for the coronaviruses).

The sections that follow explore the various pathways that lead from a laboratory accident to a laboratory acquired infection for each of the pathogens examined. The relative probability of laboratory acquired infections when working with pathogens with GoF phenotypes compared to the work with wild type pathogens is described.

6.4.4.1 Laboratory Acquired Infections and Seasonal Influenza

When working with seasonal influenza under BSL-2 conditions, the accidental generation of aerosols produces the majority of laboratory acquired infections because no personal respiratory protection is worn (and the agent is extremely infectious). Only a small minority of accidental infections are caused by the contamination of the hands. Figure 6.14 shows the various accident pathways that contribute to the probability of a laboratory acquired infection. Data in these figures comes from comparing the total frequencies of laboratory acquired infections, which in turn is derived from the predicted frequency of exposure events with various pathogen amounts as calculated by the Fault Tree Models. Comparing the 5th, 50th and 95th percentile[361] of our Monte Carlo simulations suggests that changes in risk of less than a factor of two are not that significant because the total probability of an infection from any cause changes

[361] Recall that the p50 is the median result, whereas the p95 is the result in which 95% of all results have a smaller value, and the p5 is the result in which 5% of all results have a smaller value.

by this much between the samples (relative to the p50, the p5 is 2.3-fold less and the p95 is 1.6-fold greater). In terms of the incidents that contribute to the probability of infection, the 5th and 95th percentile results are similar to the p50 but the fomite-based pathways contribute to the infections slightly more frequently (splashes cause from 0.5 to 3% of infections and cuts cause from 0.8% to 10% of infections depending on the sample).

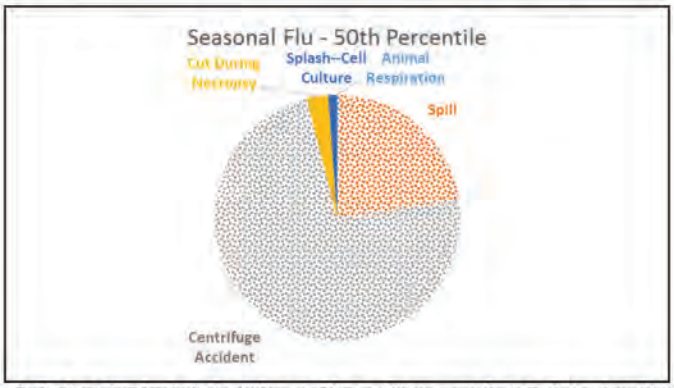

Figure 6.14. A pie chart showing how the various accident pathways contribute to the total probability of a laboratory acquired infection for seasonal influenza. Solid colored sections are fomite-based hazards, hatched sections are aerosol-based hazards and stippled sections are both fomite- and aerosol-based hazards. The median result of the Monte Carlo analysis is shown.

Some of the GoF phenotypes could affect risk of a laboratory infection (Table 6.3). Viruses that grow to a high titer could increase the dose received by a victim via an aerosol or fomite-based exposure and approximately double the chance of a laboratory acquired infection if cultures with these high titers are routinely manipulated. That being said, many strains of seasonal influenza already grow to a titer of 1E8/ml and increasing this titer may not be desirable or scientifically achievable. The strain could be made antiviral resistant, which would vitiate providing antivirals after a high-risk exposure. Similarly, the strain could be made to evade the protection afforded by vaccines. Because seasonal influenza is already adapted to humans, this GoF phenotype is not relevant for this pathogen. Table 6.3 shows the relative increase in the probability of a laboratory acquired infection predicted if modified strains of seasonal influenza are created. As titer increases, splashes begin to contribute more to the risk of a laboratory acquired infection, but still contribute less than 20% of the total risk (not shown).

Table 6.3 Increase in the Probability of a Laboratory Acquired Infection Associated with GoF Phenotypes in Seasonal Influenza	
Phenotype	**Increase in Probability of a LAI**
Evasion of vaccines	+50%
Antiviral resistance	+40%
Growth to 1E9/ml	+100%
Growth to 1E10/ml	+140%
Adaptation to humans	N/A

6.4.4.2 Laboratory Acquired Infections and Pandemic Influenza

When working with pandemic influenza under BSL-3 conditions, the accidental generation of aerosols produces the majority of laboratory acquired infections even though personal respiratory protection is worn. About 20% of accidental infections are caused by the contamination of the hands. This finding holds across the p5 and p95 samples (although in the p95 the fomite-pathways contribute to ~30% of risk) Figure 6.15 shows the various accident pathways that contribute to the probability of a laboratory acquired infection.

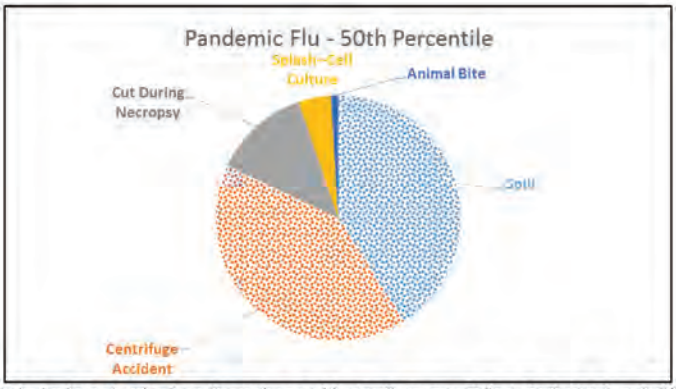

Figure 6.15. A pie chart showing how the various accident pathways contribute to the total probability of a laboratory acquired infection for pandemic influenza. Solid colored sections are fomite-based hazards, hatched sections are aerosol-based hazards and stippled sections are both fomite- and aerosol-based hazards. The median result of the Monte Carlo analysis is shown.

Some of the GoF phenotypes could affect risk of a laboratory infection. Viruses that grow to a high titer could increase the dose received by a victim via an aerosol or fomite-based exposure. The strain could be made antiviral resistant, which would vitiate providing antivirals after a high-risk exposure. Similarly, the strain could be made to evade the protection afforded by vaccines. Because pandemic influenza is already adapted to humans, this GoF phenotype is not relevant for this pathogen. Table 6.4 shows the relative increase in the probability of a laboratory acquired infection predicted if modified strains of pandemic influenza are created. Enhancing the growth of pandemic strains to achieve titers of 1E9 or 1E10/ml can significantly increase the risk that a laboratory acquired infection would occur because the exposures that drive risk are normally very low. That being said, some strains of pandemic influenza already grow to a

titer of 1E8/ml and increasing this titer may not be desirable or scientifically achievable. Increasing the maximum titer of poor growing strains to 1E8/ml simply allows these strains to approach the risk modeled for the more robust strains.

Table 6.4 Increase in the Probability of a Laboratory Acquired Infection Associated with GoF Phenotypes in Pandemic Influenza

Phenotype	Increase in Probability of a LAI
Evasion of vaccines	+50%
Antiviral resistance	+40%
Growth to 1E9/ml	+90%
Growth to 1E10/ml	+520%
Adaptation to humans	N/A

6.4.4.3 Laboratory Acquired Infections and Avian Influenza

When working with avian influenza under BSL-3 conditions, the accidental release of improperly decontaminated solid waste drives the risk of an accidental infection, albeit of a wild bird, not a human. In these cases, the operator committed an error, such as packing the autoclave too tightly with bedding-containing cages or carcasses such that the steam did not penetrate into all parts of the waste. Alternatively, the operator could run an improper cycle such that the temperature was not reached for the required length of time. The waste then enters the solid waste stream and is dumped, whereupon wild birds (like gulls that frequent garbage dumps) access the infectious material and are infected. Data is lacking to determine the percent of waste containers actually accessed by gulls, or even how an outbreak would unfold if gulls that live in garbage dumps were infected; however, the analysis assumes that an avian outbreak would occur with attendant human infections and deaths from exposure to infected wild or domestic birds. Direct infections of workers in the laboratory represent less than 25% of the probability of an infection. Figure 6.16 shows the various accident pathways that contribute to the probability of a laboratory acquired infection.

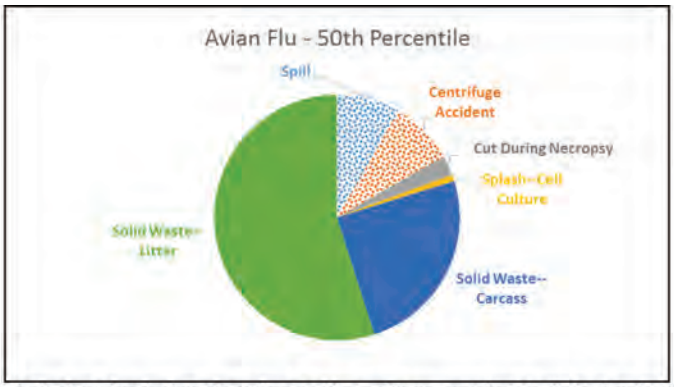

Figure 6.16. A pie chart showing how the various accident pathways contribute to the total probability of a laboratory acquired infection for avian influenza (including infections of wild birds). Both solid waste pathways infect wild birds only, and not humans. Solid colored sections are fomite-based hazards, hatched sections are aerosol-based hazards and stippled sections are both fomite- and aerosol-based hazards. The median result of the Monte Carlo analysis is shown.

The differences between the p50 and p5 result illustrate some of the significant uncertainty of the causes of accidents when working with avian influenza viruses (Figure 6.17). Although the pathways that lead to infection of a laboratory worker (compared to a bird) begin to contribute more to the probability of accidents, these pathways still contribute to a minority of infections.

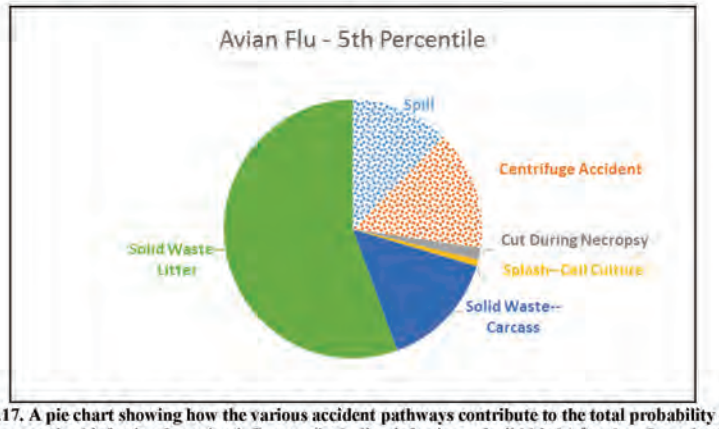

Figure 6.17. A pie chart showing how the various accident pathways contribute to the total probability of a laboratory acquired infection for avian influenza (including infections of wild birds) for the p5 result of the Monte Carlo analysis. Both solid waste pathways infect wild birds only, and not humans. Solid colored sections are fomite-based hazards, hatched sections are aerosol-based hazards and stippled sections are both fomite- and aerosol-based hazards.

Some of the GoF phenotypes could affect risk of a laboratory infection. Viruses that grow to a high titer could increase the dose received by a victim via an aerosol or fomite-based exposure. The strain could be made antiviral resistant, which would vitiate providing antivirals after a high-risk exposure. Similarly, the strain could be made to evade the protection afforded by vaccines. If the strain were adapted to humans, we assume it would poorly infect birds but would greatly decrease the infectious dose in humans. Table 6.5 shows the relative increase in the probability of a laboratory acquired infection predicted if modified strains of pandemic influenza are created. No GoF phenotype increases the risk that an accidental infection occurs with the avian influenza viruses, because so much of the risk of an accidental infection wild type pathogen is driven by the infection of birds from solid waste (all the GoF phenotypes affect the human health risk). In fact, adapting the strain to humans DECREASES the probability that a laboratory accident will lead to an infection of an animal or person by 30% because although the strain is more likely to infect a person, it is much less likely to lead to a dangerous outbreak in birds, which can sicken much more than a handful of laboratory workers. Note, because this analysis considers one GoF trait at a time, the adaptation to humans is assumed to create a strain that is more infectious in humans but not alter its transmissibility.

Table 6.5 Increase in the Probability of a Laboratory Acquired Infection Associated with GoF Phenotypes in Avian Influenza

Phenotype	Increase in Probability of a LAI
Evasion of vaccines	+11%
Antiviral resistance	+8%
Growth to 1E9/ml	+20%
Growth to 1E10/ml	+120%
Adaptation to humans	-30%

Figure 6.18 shows the accident pathways that lead to human infections for avian influenza strains adapted to infect humans (instead of birds).

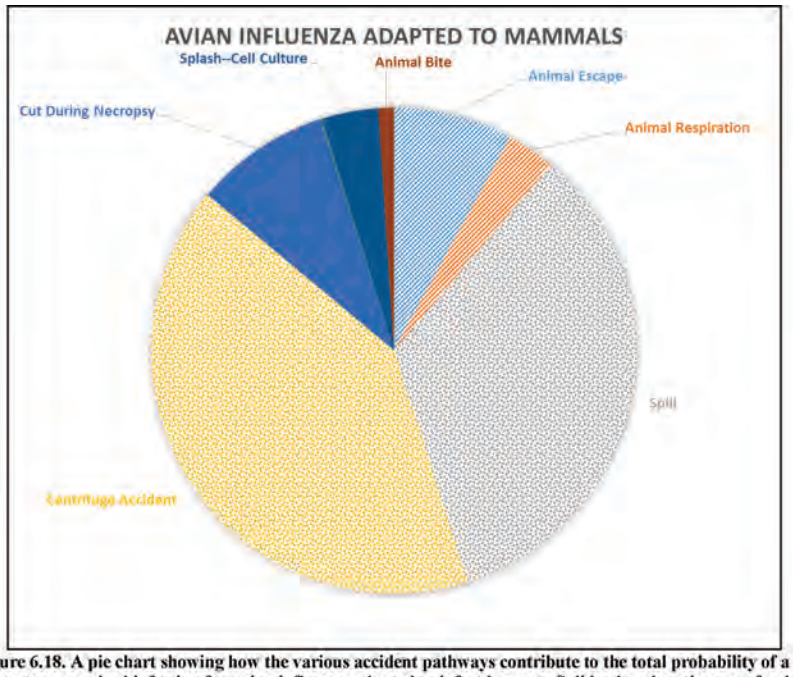

Figure 6.18. A pie chart showing how the various accident pathways contribute to the total probability of a laboratory acquired infection for avian influenza adapted to infect humans. Solid colored sections are fomite-based hazards, hatched sections are aerosol-based hazards and stippled sections are both fomite- and aerosol-based hazards. The median result of the Monte Carlo analysis is shown.

6.4.4.4 Laboratory Acquired Infections and Coronaviruses

When working with the coronaviruses under BSL-3 conditions, the accidental generation of aerosols produces the vast majority of laboratory acquired infections even though personal respiratory protection is worn. Working with infected animals poses minimal risk because mouse adapted strains poorly infect human cells due to changes in the spike protein. Figure 6.19 shows the various accident pathways that contribute to the probability of a laboratory acquired infection, the p5 and p95 results from the Monte Carlo analysis are similar (not shown).

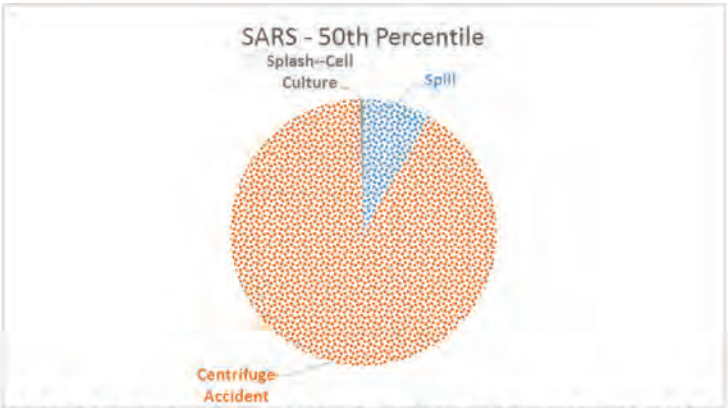

Figure 6.19. A pie chart showing how the various accident pathways contribute to the total probability of a laboratory acquired infection for SARS-CoV (the chart for MERS-CoV is very similar). Solid colored sections are fomite-based hazards, hatched sections are aerosol-based hazards and stippled sections are both fomite- and aerosol-based hazards. The median result of the Monte Carlo analysis is shown.

Only one of the GoF phenotypes could affect risk of a laboratory infection. Viruses that grow to a high titer could increase the dose received by a victim via an aerosol or fomite-based exposure. Because the coronaviruses are already adapted to humans, and because there are no countermeasures in use for protecting against infections with this pathogen, other GoF phenotypes are not relevant for this pathogen. Table 6.6 shows the relative increase in the probability of a laboratory acquired infection predicted if modified strains of the coronaviruses are created. Enhancing the growth of the coronaviruses to achieve titers of 1E9 or 1E10/ml can significantly increase the risk that a laboratory acquired infection would occur because the exposures that drive risk are normally very low. Under these circumstances, contamination of the hands beings to significantly drive risk, growing to cause about 20% of all laboratory infections for strains that grow to 1E10/ml (not shown). That being said, SARS- and MERS-CoV already grow to a titer of 1E8/ml, and increasing this titer may not be desirable or scientifically achievable.

Table 6.6 Increase in the Probability of a Laboratory Acquired Infection Associated with GoF Phenotypes in the Coronaviruses

Phenotype	Increase in Probability of a LAI
Evasion of vaccines	N/A
Antiviral resistance	N/A
Growth to 1E9/ml	+260% (SARS-CoV), +160% MERS-CoV
Growth to 1E10/ml	+860 (SARS-CoV), +550% (MERS-CoV)
Adaptation to humans	N/A
**N/A marks a phenotype not applicable to the coronaviruses*	

6.4.4.5 Effect of various research conditions on risk on probability of loss of containment

6.4.4.5.1 Effect of changing the biosafety level when working with GoF pathogens

This section describes how changing the biosafety level of the laboratory in which GoF pathogens are manipulated changes risk. All GoF pathogens except for seasonal influenza are manipulated at BSL-3 containment at least. Increasing the containment level of seasonal influenza decreases the probability of a laboratory acquired infection by three-fold, which, notably, would partially compensate for the increases in risk caused by the riskiest GoF phenotypes. For all but avian influenza, the increase or decrease in risk is caused by the addition or elimination of personal respiratory protection (such as PAPRs). For avian influenza, fewer mistakes need to be committed to release infected solid waste at BSL-2 than BSL-3 leading to an increase in the frequency of infections of wild birds.

Table 6.7. Change in the Probability of a Laboratory Acquired Infection (LAI) for Changes in the Containment Level Required for Manipulating the GoF Pathogens

Pathogen	Change in BSL	Change in Probability of an LAI
Seasonal influenza	Increase from BSL-2 to BSL-3	3-fold decrease
Pandemic influenza	Decrease from BSL-3 to BSL-2	3.5-fold increase
Avian influenza	Decrease from BSL-3 to BSL-2	110-fold increase
SARS-CoV	Decrease from BSL-3 to BSL-2	Less than 2-fold increase
MERS-CoV	Decrease from BSL-3 to BSL-2	Less than 2-fold increase

In contrast, if any of the other GoF pathogens were manipulated under BSL-2 conditions instead of BSL-3, the probability of a laboratory acquired infection would, unsurprisingly, increase, although this increase is small for the coronaviruses. This analysis suggests that work on influenza viruses in parts of the world with less stringent biosafety standards than the US could be expected to have up to an order of magnitude more accidents resulting in an infection.

6.4.4.5.2 Factors That Influence the Probability of Accidents with Seasonal Influenza Virus

To understand which laboratory features and practices influence the probability of a laboratory acquired infection with the risk of causing an outbreak, a sensitivity analysis was performed in which the values of any parameter were set to the lowest or highest level while all other parameter values were allowed to vary as normal. The results of this sensitivity analysis for seasonal influenza at BSL-2 are shown in the one-sided tornado plot in Figure 6.20 wherein the width of the boxes shows the increase in the probability of an infection if the parameter is set from its value that minimizes the probability to the value that maximizes it.

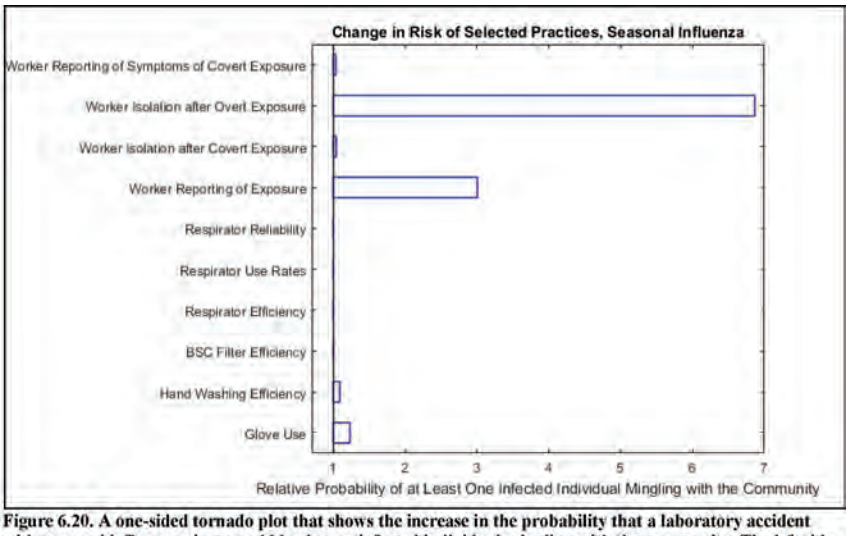

Figure 6.20. A one-sided tornado plot that shows the increase in the probability that a laboratory accident with seasonal influenza virus would lead to an infected individual mingling with the community. The left side of each box (set to one) represents that value of that parameter that minimizes this probability, whereas the right side is the value that maximizes this probability.

The most influential features that influence the risk of an infection occurring and that worker posing a risk to the community is the behavior of that worker. Maximizing the probability that a worker will not properly report a high-risk exposure can increase the probability of a dangerous infection by a three-fold. Similarly, maximizing the chance that a worker violates isolation protocols after an overt exposure can increase risk by seven-fold. For this reason, extensive training on the benefit of reporting, health monitoring and isolation could increase compliance and greatly reduce risk. No other parameter is very influential (partially because respirators are not worn in BSL-2).

6.4.4.5.3 Factors That Influence the Probability of Accidents with Pandemic Influenza Virus

The results of the sensitivity analysis for pandemic influenza at BSL-3 are shown in the one-sided tornado plot in Figure 6.21. The width of the boxes shows the increase in the probability of an infection if the parameter is set from its value that minimizes the probability to the value that maximizes it.

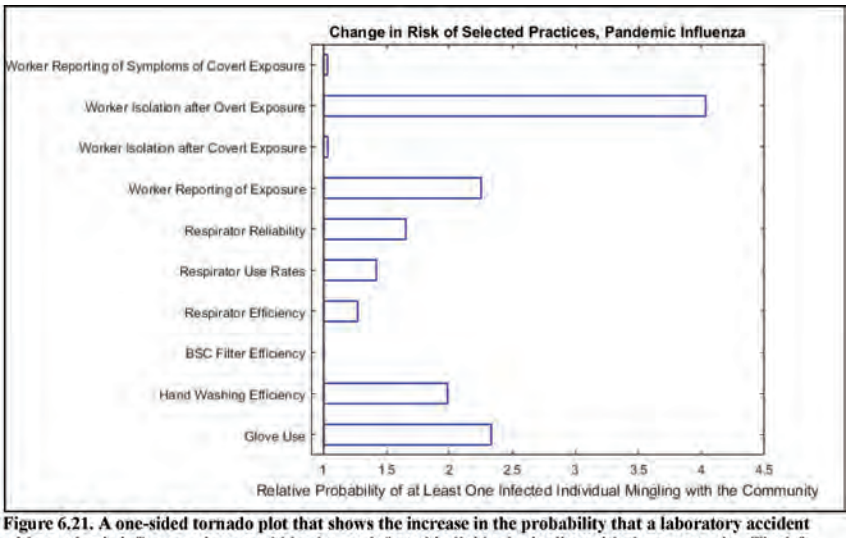

Figure 6.21. A one-sided tornado plot that shows the increase in the probability that a laboratory accident with pandemic influenza virus would lead to an infected individual mingling with the community. The left side of each box (set to one) represents that value of that parameter that minimizes this probability, whereas the right side is the value that maximizes this probability.

The most important practice of reducing the probability of a dangerous infection with pandemic influenza is the isolation of possibly infected workers (poor isolation practices increase the risk of an infection by four-fold). Similarly, poor reporting of exposure can more than double the probability of a double infection. Failure to double glove can more than double the probability of an infection, whereas poorly functioning or fitted respirators can nearly double this probability.

6.4.4.5.4 Factors That Influence the Probability of Accidents with Avian Influenza Virus

The results of the sensitivity analysis for avian influenza at BSL-3 are shown in the one-sided tornado plot in Figure 6.22 wherein the width of the boxes shows the increase in the probability of an infection if the parameter is set from its value that minimizes the probability to the value that maximizes it. No feature or practice in the assessment conducted influences the probability of a dangerous infection by more than 1.5-fold, which makes sense because most of the risk is driven by errors in solid waste processing.

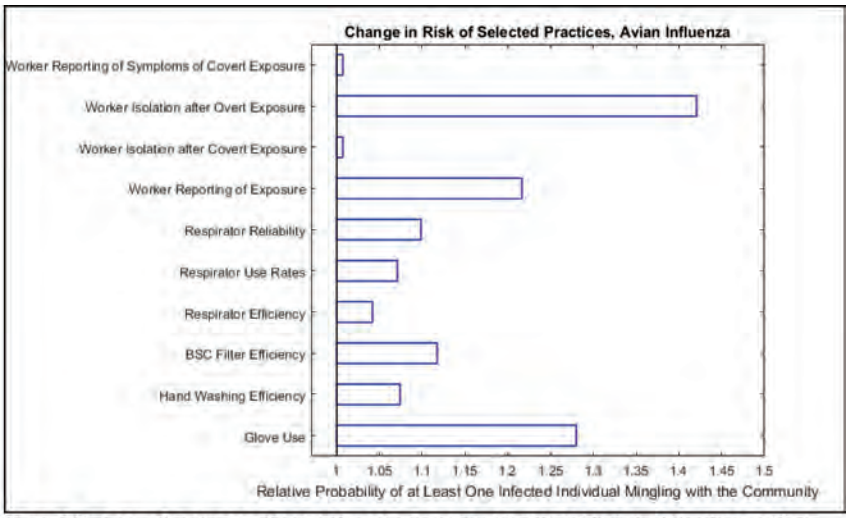

Figure 6.22. A one-sided tornado plot that shows the increase in the probability that a laboratory accident with avian influenza virus would lead to an infected individual mingling with the community. The left side of each box (set to one) represents that value of that parameter that minimizes this probability, whereas the right side is the value that maximizes this probability.

6.4.4.5.5 Factors That Influence the Probability of Accidents with Coronaviruses

The results of the sensitivity analysis for the coronaviruses at BSL-3 are shown in the one-sided tornado plot in Figure 6.23 wherein the width of the boxes shows the increase in the probability of an infection if the parameter is set from its value that minimizes the probability to the value that maximizes it.

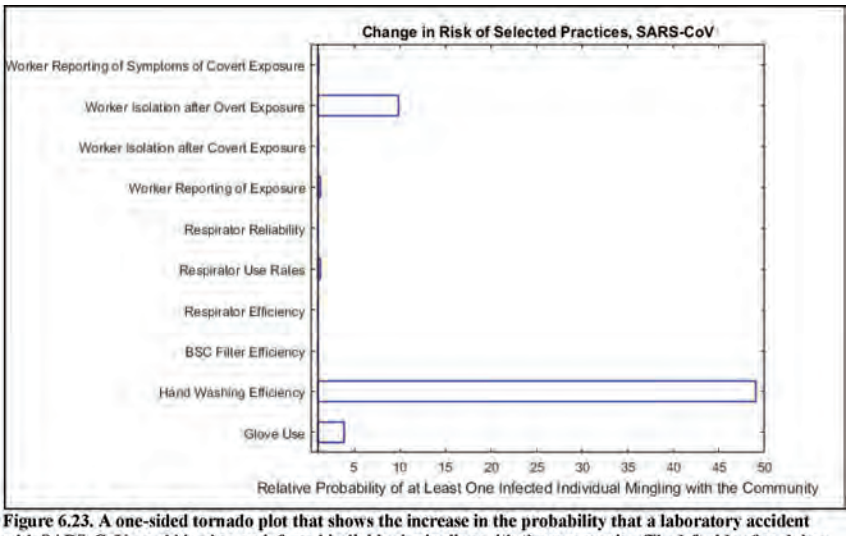

Figure 6.23. A one-sided tornado plot that shows the increase in the probability that a laboratory accident with SARS-CoV would lead to an infected individual mingling with the community. The left side of each box (set to one) represents that value of that parameter that minimizes this probability, whereas the right side is the value that maximizes this probability.

Three practices have a significant influence on the probability that an infection occurs and the worker mingles with the community. Firstly, failure to double glove can increase the probability by up to four-fold. From the same exposure pathways, poor hand washing can increase the probability by nearly 50-fold. These findings demonstrate that worker education and training on proper techniques for reducing hand contamination may significantly reduce risk of working with the coronaviruses. Also, poor adherence to isolation protocols can increase the probability that an infected worker mingles with the population by ten-fold. Once again, training on the importance of health monitoring and isolation could greatly reduce risk.

The results for MERS-CoV follow the same overall trends, and are shown in Figure 6.24, below.

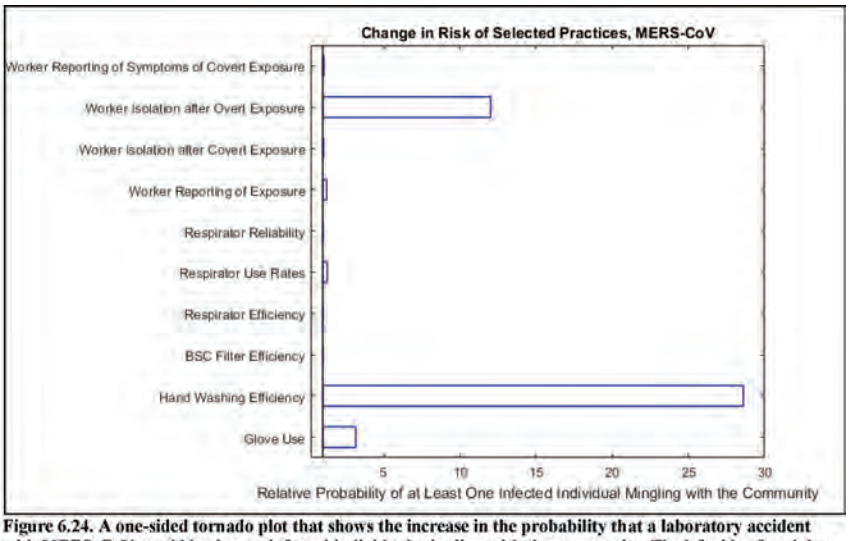

Figure 6.24. A one-sided tornado plot that shows the increase in the probability that a laboratory accident with MERS-CoV would lead to an infected individual mingling with the community. The left side of each box (set to one) represents that value of that parameter that minimizes this probability, whereas the right side is the value that maximizes this probability.

6.4.4.5.6 Importance of Laboratory Worker Training

By far, the most critical driver of the probability of a dangerous infection is the behavior of workers themselves. As discussed above, the probability that a worker reports a high risk exposure or adheres to isolation protocols can significantly influence the probability that an infected worker would mingle with the population. However, the probability that a worker would carelessly or forgetfully cause the incident in the laboratory is the most influential factor on risk. Figure 6.25 shows the relative influence of parameters influencing human error rates in the laboratory against all other parameters investigated. In this instance, all nodes in the fault trees that were based on the probability of a human error occurring had their failure probabilities (i.e., the probability a mistake is committed) simultaneously set to their maximum or minimum values. Only human errors that occur within the laboratory leading to an accident were considered; the probabilities of human errors occurring after an incident occurs, such as failures to report incidents or remain in isolation, were unchanged in this analysis. From this figure, human error rates can influence the probability of an infection by more than 100-fold (whereas the next most influential parameter for seasonal influenza changes this probability by nearly tenfold). Across all pathogens studied, human error rates in the laboratory this type of parameter influence the probability of a dangerous infection from 100-1,000-fold (data not shown).

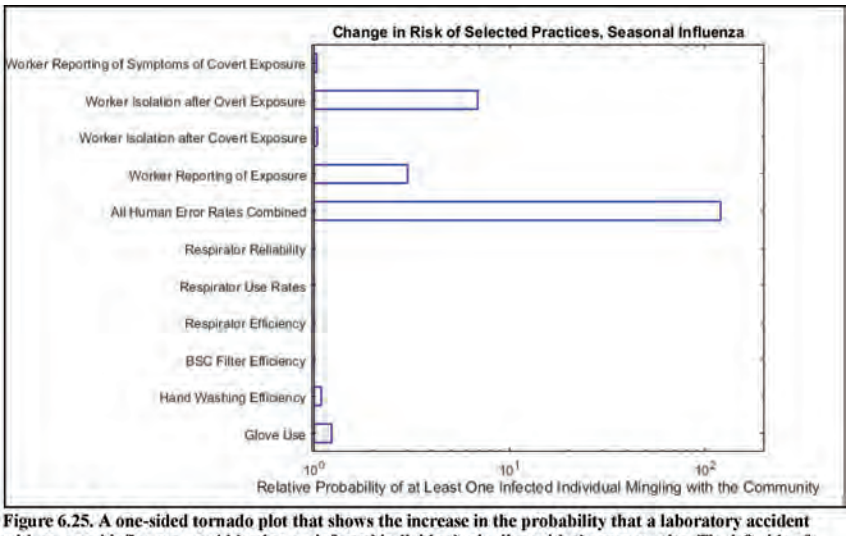

Figure 6.25. A one-sided tornado plot that shows the increase in the probability that a laboratory accident with seasonal influenza would lead to an infected individual mingling with the community. The left side of each box (set to one) represents that value of that parameter that minimizes this probability, whereas the right side is the value that maximizes this probability. The x-axis is on a log scale.

This analysis reflects both aleatoric and epistemic uncertainty. That is, data are lacking on how often humans will make mistakes of a variety of kinds in a biological laboratory (epistemic uncertainty). However, even if relevant observational data were available to inform these human error rates, significant aleatoric uncertainty would remain. That is, laboratory workers are humans and some humans are more prone than others to errors due to carelessness, unfamiliarity with protocols, distraction, or stress. Many who have experience working in a microbiology laboratory could identify co-workers with whom no one would share reagents due to the perception that the co-worker would contaminate or otherwise compromise the reagent. Aleatoric uncertainty will always exist because at any given time, it is unknown which type of person will be working in the laboratory (and what stresses they will be under). This analysis suggests that efforts to reduce stressors on workers could significantly improve laboratory safety. Also, measures to identify and re-train workers that are prone to carelessness or forgetfulness may have similar benefits. Moreover, as described by the laboratory safety stakeholders we interviewed, efforts to "train-in" to a BSL-3 laboratory by first demonstrating competence and mastery of protocols in a BSL-2 laboratory could significantly improve safety. Lastly, some stakeholders mentioned that dedicated professionals handle some sensitive laboratory protocols, such as the operation of autoclaves, to reduce the probability of the release of contaminated materials. Such practices would also significantly improve safety.

6.5 Consequences of an Outbreak Caused by an Avian Influenza Strain That Is Not Transmissible in Mammals

As discussed in the methods, we are unable to adequately model the human health consequences of an outbreak of an influenza virus that is not transmissible amongst people but is maintained in birds. Our simple models, based on the characteristics of past avian influenza outbreaks, suggest that an average of

100 people would die and 1,000 people would be clinically ill from contact with infected wild birds or poultry.

Most GoF phenotypes would not affect risk (clearly, if the strain were made transmissible in mammals, risk could change greatly as explored in Section 6.7 below). Enhanced growth in culture would not affect the outbreak unless this trait was related to pathogenicity or infectiousness. Ability to overcome immunity would not increase risk because most humans have no prior immunity from exposure to avian strains and novel vaccines are not stockpiled in quantity for an outbreak of influenza that is not human transmissible. Resistance to antivirals is of minimal risk because some wild type strains of avian influenza are already resistant and antivirals at most would reduce the number of deaths by half (and the role of antivirals in preventing onward transmission is moot).

No prediction was able to be made on how adaptation to a mammalian host, which could reduce the median infectious dose, affects risk. The infectious dose of any given strain of avian influenza in humans is unknown as is the dose to which past victims had been exposed to. It is possible that upon exposure to an infected bird, a human receives either a large dose or no dose at all (if, for example, infection is generally caused by inoculation with a globule of infected feces that could contain billions of active virus particles). In this case, a reduction of the median infectious dose would minimally affect risk. Conversely, many people could be exposed to very low doses and not become infected; if the infectious dose decreased perhaps all these people would develop illness.

In a simple way, the effect of an increased case fatality rate on risk can be made. Figure 6.26 shows the human deaths predicted to occur from an outbreak of wild type avian influenza, an outbreak caused by a strain that causes an illness with half the rate of survival of wild type avian influenza, and an outbreak that causes an illness caused by a strain with quarter the rate of survival of wild type avian influenza. Decreases in rates of survival must be modeled instead of increases in death rates because the fatality rate could be as high as 50% in some wild type strains. Outbreaks of wild type avian influenza are predicted to cause about 100 deaths, and very few outbreaks would cause up to 500 deaths. If a strain were modified to decrease the survival rate in victims by half, the outbreaks cause about 300 deaths on average, but up to 700 deaths in rare cases. If a strain were modified to decrease the survival rate in victims by a quarter, the outbreaks cause about 500 deaths on average, but up to 900 deaths in rare cases. This analysis presumes that the most pathogenic strains of avian influenza have an inflated case fatality rate due to the under-reporting of mild cases. If the case fatality rate of the most pathogenic strains of avian influenza is truly 50% then increasing this trait would not make the strain much more dangerous.

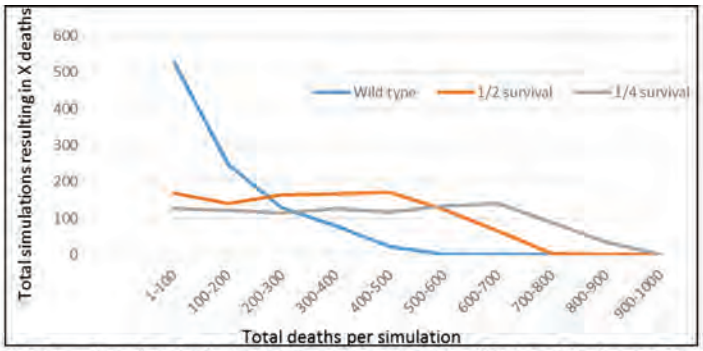

Figure 6.26. The number of outbreak simulations (out of 1,000 per condition) resulting in a number of deaths for wild type avian influenza and strains modified to be more pathogenic. Outbreaks of wild type avian influenza are in blue, outbreaks of a strain that causes disease with half the survival rate of wild type avian influenza are in orange, and outbreaks of a strain that causes disease with quarter the survival rate of wild type avian influenza are in grey.

That being said, the state of modeling of avian influenza outbreaks in human populations is very rudimentary, so confident predictions of the risk of modified avian influenza strains in human populations is currently impossible. Additional data on the risk factors that lead to human infection, the life cycle of disease in its various avian hosts and factors that relate the biology of the virus to the pathology in the various hosts is needed to improve modeling of this infectious disease.

6.6 Risk of an Outbreak Escaping Local Control of Pathogens That Are Transmissible in Mammals

After an outbreak is initiated, the GoF phenotype of enhanced growth characteristics in culture or eggs no longer has any influence on risk. If this phenotype also increases transmissibility in humans, then the models capture changes in risk through those parameters. Pathogenicity is indirectly captured in the probability that an outbreak escapes local control. That is, the strength of public health control measures and social distancing exerts a critical influence on the probability that an outbreak escapes local control, which is assumed to be stronger when the outbreak is causing significant mortality than when the outbreak resembles a typical influenza season. Pathogenicity directly influences consequences in terms of the number of deaths that occur should an outbreak not escape local control (if the outbreak escapes, then the consequences will be dominated by the global deaths summed across all regions, not the deaths in one community). Ability to overcome immunity induced by vaccination is not relevant because a matched vaccine will not be available in quantity in time to respond to the initial outbreak.

Antivirals have never been dispensed to address a nascent outbreak of influenza, and public health authorities interviewed have no concrete plans for the use of antivirals in an outbreak arising from a local laboratory. For this reason, we do not know if, in the case of a laboratory-associated outbreak, antivirals would be mass dispensed to the entire outbreak area, if they would be distributed to all contacts of an infected person, or if they would just be given to the infected individuals. Moreover, some strains of influenza are naturally resistant to antivirals, and we do not know which strain would be involved in an outbreak. For these reasons, antivirals were not included in the branching process model. Because data exists on how antivirals are used in the context of an ongoing global pandemic of influenza, antivirals are included in the global influenza models described in Section 6.11, below. Similarly, antivirals can be

given upon high-risk exposures in the laboratory to prevent the onset of illness or reduce transmissibility if an infection occurs, as described in Section 6.4.

All of the figures shown assume that just one person is initially infected. These events dominate risk because they are much more likely to occur and have similar consequences to events that initially infect multiple people. As discussed in Figure 6.27 below, increasing the number of initially infected at most increases the probability of a global pandemic by ten-fold. However, events that lead to a single initial infection are more than 100-fold more likely to occur.

Even if an infected person mingles with the local population, secondary infections in the population are not guaranteed. In fact, for some poorly transmissible pathogens (or the coronaviruses that have a high variance in transmissibility), in most cases no secondary cases are caused just by chance. Figure 6.27 shows the relationship between transmissibility and the percent of outbreaks that create at least one secondary infection for the influenza viruses and the coronaviruses. When a single person infected with seasonal influenza mingles with the population, another person is infected just half the time (and this probability increases modestly as R_0 increases). In contrast, when a single person infected with a SARS-like disease mingles with the population, at least one secondary case is caused only 30% of the time, which is expected given the high variance of the transmissibility of that disease. If two infected people mingle with the population, the chances that at least one secondary infection is caused increases. Perhaps most importantly, as transmissibility increases dramatically, the probability of at least one secondary infection increases only modestly, by less 15% for an increase in R_0 of one (except for very low values of R_0). The chance that an infected person does not cause any secondary infections is integrated into the analysis of outbreaks escaping local control described below.

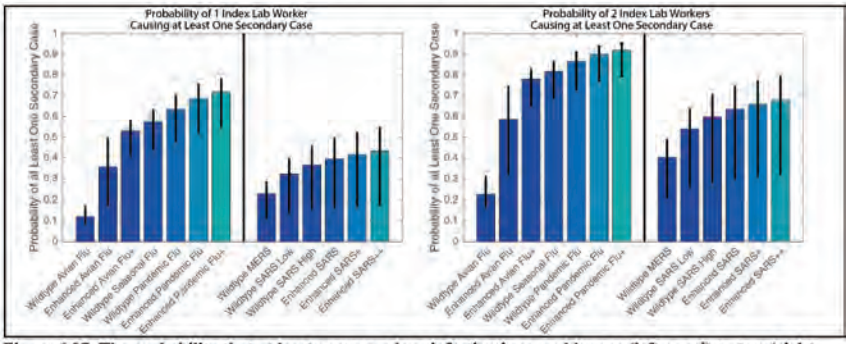

Figure 6.27. The probability that at least one secondary infection is caused by one (left panel) or two (right panel) infected people mingling with the population for various wild type and enhanced viruses. The R_0s used in this figure are 0.1-0.2 for wild type avian influenza, 0.2-1.0 for enhanced avian influenza, 1.0-1.2 for enhanced avian influenza+, 1.2-1.4 for wild type seasonal influenza (this value also captures 1918 H1N1 pandemic influenza in a modern population), 1.4-1.9 for wild type pandemic influenza (specifically strains for which our population has little residual immunity), 1.9-2.2 for enhanced pandemic influenza, 2.2-2.5 for enhanced pandemic influenza+, 0.4-0.6 for wild type MERS-CoV, 0.8-1.2 for wild type SARS-CoV low, 1.2-1.6 for wild type SARS-CoV high, 1.6-1.9 enhanced SARS-CoV, 1.9-2.2 enhanced SARS-CoV+, 2.2-2.5 for enhanced SARS-CoV++. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented). In this figure, "pandemic flu" is used to describe those pandemic influenza strains against which the population has little immunological memory (e.g., 1957 H2N2 pdm) whereas 1918 H1N1 pdm is as transmissible as a seasonal influenza strain due to recent exposure of the population to the 2009 H1N1 pdm and more recent

seasonal strains (See Supplemental Information - Protection against Infection with 1918 H1N1 Pandemic Strain).

6.6.1 Effect of Enhanced Transmissibility in Mammals on Risk of an Outbreak Escaping Local Control

Transmissibility has a significant influence on the chance that an outbreak would escape local control for all GoF pathogens.

6.6.1.1 Seasonal Influenza

If a single person is initially infected by a loss of containment event with a seasonal influenza strain that has not circulated recently and the infected person mingles with the general population, stochastic forces, and control measures still cause the outbreak to extinguish the vast majority of the time. The tornado plot in Figure 6.28 illustrates how a variety of parameters influence the probability that an outbreak would escape local containment (the wider the box, the more influence that parameter value has on the outbreak escaping local control). This figure shows that an outbreak caused by a single infection with wild type seasonal influenza virus has a 20% chance of escaping local control. Of wild type strains, those with the highest R_0 values (1.4) have up to a 30% chance of escaping local control. If transmissibility were increased even further (to 2.2), the probability of an outbreak escaping local control could more than double to 60%.

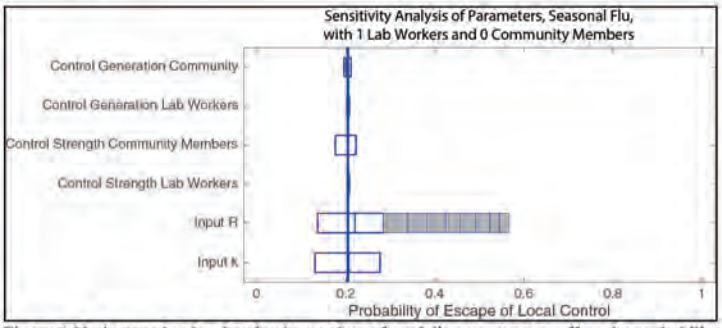

Figure 6.28. A tornado plot showing how values of modeling parameters affect the probability that an outbreak of seasonal influenza would escape local control if a single person were initially infected (shown on the Y-axis). Open boxes represent the range of probabilities that an outbreak would escape local control for all parameter values sampled for the wild type pathogen whereas grey boxes represent possible enhancements in a GoF strain. The vertical line shows the median result across all parameter values for an outbreak caused by a wild type strain.

Although the probability of an outbreak escaping local control is sensitive to the transmissibility of the strain that causes the outbreak, this increase poses a risk only if the creation of a strain with such properties is feasible. Figure 6.29 shows the relationship between the transmissibility of a seasonal influenza strain and the probability that a resulting outbreak would escape local control. These data show that modifying a wild type seasonal influenza strain associated with an average R_0 value (1.3) so that it has the transmissibility associated with an average pandemic influenza strain (1.7) doubles the probability that an outbreak would escape local control. Also of note, the least transmissible wild type strains (R_0 of

1.2) are roughly two-to-three-fold less likely to cause an outbreak that escapes local control as the most transmissible wild type strains (R_0 of 1.4).

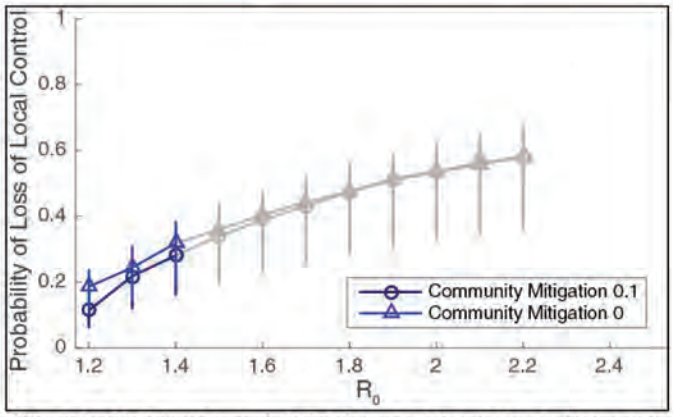

Figure 6.29. The relationship between transmissibility of seasonal influenza virus (as measured by the R_0 of the resulting outbreak) and the probability that an outbreak escapes local control. Grey represents various manipulations to increase the transmissibility of the virus beyond estimates for wild type strains. The colors correspond to those represented in the tornado plot in Figure 6.28. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented). The error bars reflect the effect of an uncertain k value (the variance in transmissibility of the disease among those infected) on the probability that an outbreak escapes local control. For this reason, for any particular k value, the relative increase in the probability that an outbreak escapes local should mirror the shape of the line given for the median result.

6.6.1.2 Pandemic Influenza

If a single person is initially infected by a loss of containment event with a pandemic influenza strain and that infected person mingles with the general population, stochastic forces, and control measures still cause the outbreak to extinguish the majority of the time. Figure 6.30 shows that an outbreak caused by a single infection with wild type pandemic influenza virus has a 20% chance of escaping local control (because the transmissibility of 1918 H1N1 pdm is less than that of new seasonal strains due to recent exposure to 2009 H1N1 pdm, see Supplemental Information - Protection against Infection with 1918 H1N1 Pandemic Strain). However, our population has little residual immunity against the H2 pandemic strains, so some wild type pandemic strains have up to a 30% chance of escaping local control (the rightmost portion of the open box). If transmissibility were increased even further (to 2.5), the probability of an outbreak escaping local control could more than double to 50%.

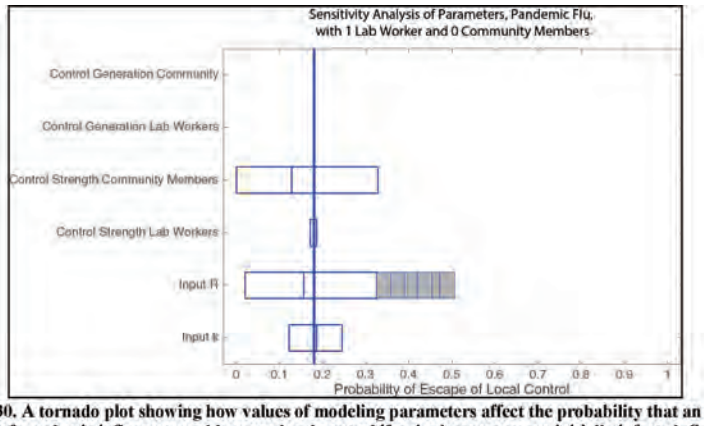

Figure 6.30. A tornado plot showing how values of modeling parameters affect the probability that an outbreak of pandemic influenza would escape local control if a single person were initially infected. Open boxes represent the range of probabilities that an outbreak would escape local control for all parameter values sampled for the wild type pathogen whereas grey boxes represent possible enhancements in a GoF strain. The vertical line shows the median result across all parameter values for an outbreak caused by a wild type strain.

Figure 6.31 shows the relationship between the transmissibility of a pandemic influenza strain and the probability that a resulting outbreak would escape local control. These data show that modifying a wild type pandemic influenza strain associated with an R_0 value of a strain against which little population immunity exists (like H2 strains, R_0 of 1.7) so that it has a transmissibility greater than any estimate for any influenza strain (2.4) merely increases the probability that outbreak escapes control by 50%. If, however, the local population can sustain robust social distancing throughout the nascent outbreak (for example, by reducing the number of human contacts they have by half, shown as a community mitigation of 0.5 in the figure below), these extreme R_0 values would be *required* for the outbreak to have any chance of escaping local control. This finding is intuitively obvious because halving all contacts would reduce an R_0 value of two to an R_0 of one, which is required for the outbreak to be self-sustaining. It should be noted, however, that no experiment performed to date has increased the transmissibility of an influenza strain more than the most highly transmissible strains, and it is unknown if this result is even feasible. In contrast, increasing the transmissibility of a poorly transmissible strain (like 1918 H1N1 pdm, which, due to recent population exposure to antigenically similar H1N1 strains, has a R0 closer to 1.2), can more than double the probability of an outbreak even if little community mitigation is assumed. Increasing the transmissibility to that of other pandemic strains (by, for example, changing its antigenic properties) would double the probability of escape, and increasing the transmissibility further could triple the chance of escape.

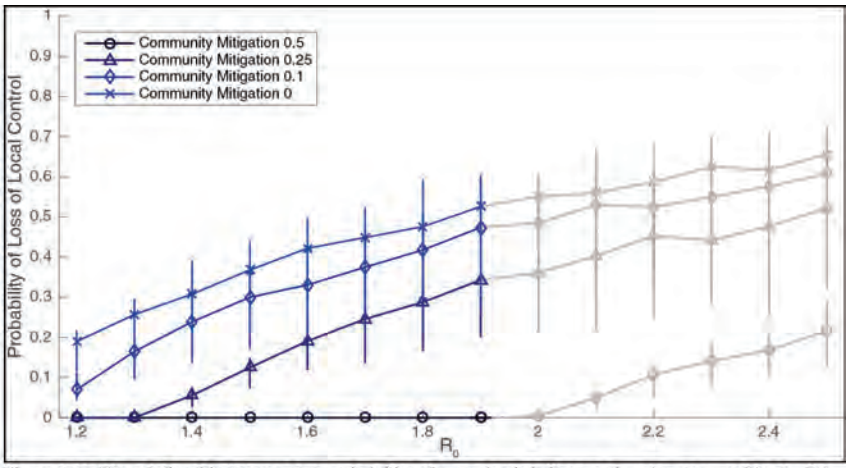

Figure 6.31. The relationship between transmissibility of a pandemic influenza virus (as measured by the R0 of the resulting outbreak) and the probability that an outbreak escapes local control. Grey indicates various manipulations to increase the transmissibility of the virus beyond estimates for wild type strains. The colors correspond to those represented in the tornado plot in Figure 6.30. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented). The error bars reflect the effect of an uncertain k value (the variance in transmissibility of the disease among those infected) on the probability that an outbreak escapes local control. For this reason, for any particular k value, the relative increase in the probability that an outbreak escapes local should mirror the shape of the line given for the median result.

6.6.1.3 Avian Influenza

Wild type avian influenza virus is insufficiently transmissible in mammals to cause an outbreak that escapes local control. Figure 6.32 shows how transmissible in people a modified strain of avian influenza would have to be to escape local control should one laboratorian be initially infected and mingle with the general population. Unless robust social distancing measures can be implemented throughout the outbreak (community mitigation 0.5 in the figure below), increasing the transmissibility of an avian influenza strain in humans to that of seasonal influenza would lead to a local outbreak with about a 10-20% chance of escaping local control. Given that the wild type strain has no chance of creating an outbreak that escapes local control (or even one that is made modestly more transmissible) this increase is extremely significant.

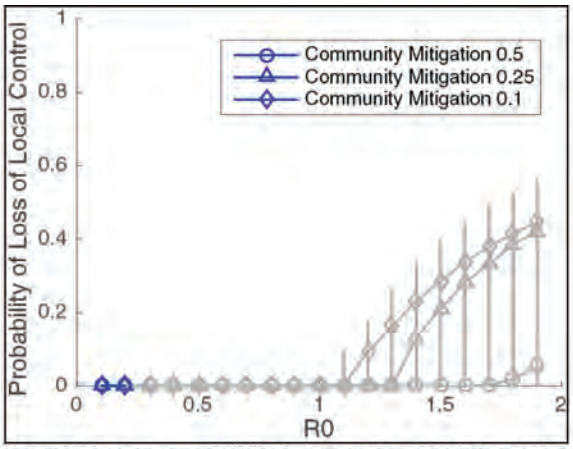

Figure 6.32. The relationship between transmissibility in humans of an avian influenza virus (as measured by the R0 of the resulting outbreak) and the probability that an outbreak escapes local control. Grey points indicate various manipulations to increase the transmissibility of the virus beyond estimates for wild type strains. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented). The error bars reflect the effect of an uncertain k value (the variance in transmissibility of the disease among those infected) on the probability that an outbreak escapes local control. For this reason, for any particular k value, the relative increase in the probability that an outbreak escapes local should mirror the shape of the line given for the median result.

6.6.1.4 Coronaviruses

If a single person is initially infected by a loss of containment event with SARS-CoV and that person mingles with the general population, stochastic forces and control measures still cause the outbreak to extinguish. Figure 6.33 shows that an outbreak caused by a single infection with wild type SARS-CoV has nearly no chance of escaping local control. The historical outbreaks of coronaviruses reinforce this finding because although these outbreaks lead to infections in several locations, they did not initiate a global pandemic because local control of the outbreak was successful in every outbreak location. As described in the Supplemental Information, most researchers consider the highest estimates for the value of R_0 to be 1.6 for outbreaks caused by wild type SARS-CoV. This value is useful for a biosafety analysis because it automatically considers the spontaneous, uncoordinated control measures that would occur until the outbreak is identified. Some researchers have estimated the R_0 to be as great as 3.0 if only the absolute earliest stage of the outbreak is considered, the strictest meaning of the term R_0.[362, 363] For our analysis, we have restricted "wild-type" SARS-CoV to R_0 values of 1.6 or less, but we also describe how a higher baseline R_0 value affects risk.

[362] Lipsitch, M., et al., Transmission dynamics and control of severe acute respiratory syndrome. Science, 2003. 300(5627): p. 1966-70.

[363] Wallinga, J. and P. Teunis, Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures. Am J Epidemiol, 2004. 160(6): p. 509-16.

As figure 6.33 shows, wild type SARS-CoV have nearly no chance of escaping local control. If we assume that community mitigation is poor, some outbreaks have up to a 10% chance of escaping local control.

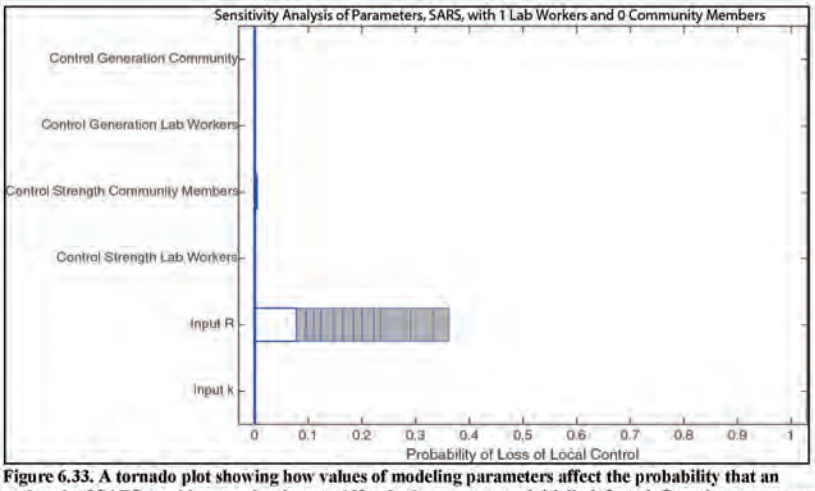

Figure 6.33. A tornado plot showing how values of modeling parameters affect the probability that an outbreak of SARS would escape local control if a single person were initially infected. Open boxes represent the range of probabilities that an outbreak would escape local control for all parameter values sampled for the wild type pathogen (assuming the R_0 value does not exceed 1.6) whereas grey boxes represent possible enhancements in a GoF strain (or greater values for the R_0 for the wild type). The vertical line shows the median result across all parameter values for an outbreak caused by a wild type strain.

Wild type MERS-CoV is not transmissible enough to cause an outbreak to escape local control. Should a MERS-CoV be modified to be as transmissible as SARS-CoV, then its probability of escaping local control would be similar.

As transmissibility of SARS-CoV increases, the probability that an outbreak escapes local control increases. The relationship between transmissibility and probability of an outbreak escaping is shown in Figure 6.34. As mentioned above, wild-type SARS-CoV has only a 10% chance of escaping local control if poor community control is assumed. If transmissibility were increased (to an R_0 of 3), the probability of an outbreak escaping local control could increase significantly to 30%. Increasing the transmissibility beyond 3.0 to 4.0 has a modest effect on the probability of escape (increasing from 30% to 38%, or roughly by 30%). In short, if the R_0 value of wild-type SARS-CoV is low (R_0 of 1.6 or less) then increasing this value can significantly increase risk. If the R_0 value of wild-type SARS-CoV is already great (R_0 of 3.0) then further increases do little to increase risk. The relationship for MERS-CoV is similar to that shown in Figure 6.34 except that GoF experiments that increase transmissibility must be conducted for the pathogen to have any chance of creating an outbreak that escapes local control.

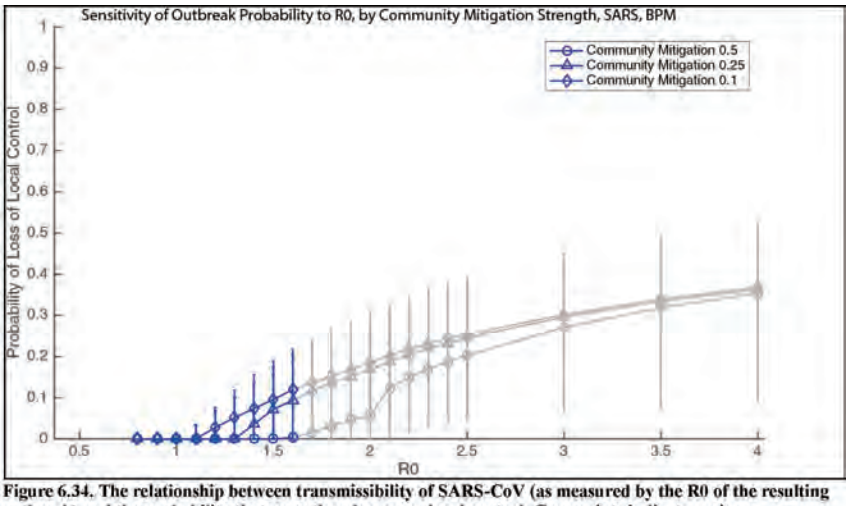

Figure 6.34. The relationship between transmissibility of SARS-CoV (as measured by the R0 of the resulting outbreak) and the probability that an outbreak escapes local control. Grey points indicate various manipulations to increase the transmissibility of SARS-CoV in humans beyond the highest estimates for the first few generations of infections caused by the virus (R_0=1.6). We here show the probability of escape of a SARS-CoV with an R_0 of 3.0 as well. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.6.2 Effect of Enhanced Pathogenicity on Risk of an Outbreak Escaping Local Control

6.6.2.1 Pandemic/Seasonal Influenza

All influenza outbreaks that extinguish do so when the outbreak is relatively small. For this reason, even the most highly pathogenic strains influenza would lead to only a handful of deaths (we predict that even a 1918-like strain would not result in, on average, even one fatality if the outbreak extinguished locally). If the outbreak escaped local control and spread throughout the world, vastly more deaths could occur, but these consequences are assessed in Section 6.11.

Interestingly, an outbreak associated with significant mortality may trigger more robust and prolonged social distancing, which would greatly decrease the chance that an outbreak would spread beyond local control. For this reason, an outbreak caused by a strain that is modified to be more deadly may actually reduce risk, although we cannot quantify how the public will react to a novel outbreak.

6.6.2.2 Avian Influenza

Wild type avian influenza strains are already associated with a high case-fatality rate and so increasing this rate would probably have little influence on the robustness of a public health response. Moreover, wild type avian influenza strains are insufficiently transmissible in humans to cause an outbreak that would escape local control.

6.6.2.3 Coronaviruses

Infections with wild type SARS- and MERS-CoV is already associated with a relatively high case fatality rate. Increasing this rate is likely to have little influence on the robustness of social distancing. Also, because the case fatality rate is already significant, increasing this rate has little influence on the number of deaths expected. For SARS and MERS outbreaks that start with one person and extinguish locally, we expect less than ten people to die even if the strain were modified to be more pathogenic.

6.6.3 Effect of Overcoming/Evading Natural/Residual/Innate Immunity on the Probability of an Outbreak Escaping Local Control

6.6.3.1 Pandemic/Seasonal Influenza

Innate/residual immunity in a population can significantly affect the kinetics of an outbreak of influenza because prior exposure to recently circulating strains of influenza affords protection against similar serotypes. The protective value of residual/innate immunity is already accounted for in the effective R_0, which is one reason why the R0 for seasonal influenza is significantly less than that of pandemic influenza strains that have not circulate recently (like H2 strains). Figure 6.35, below, shows the relation between reduction in innate or residual immunity and the probability that an outbreak would escape local control for given prior immunity values.

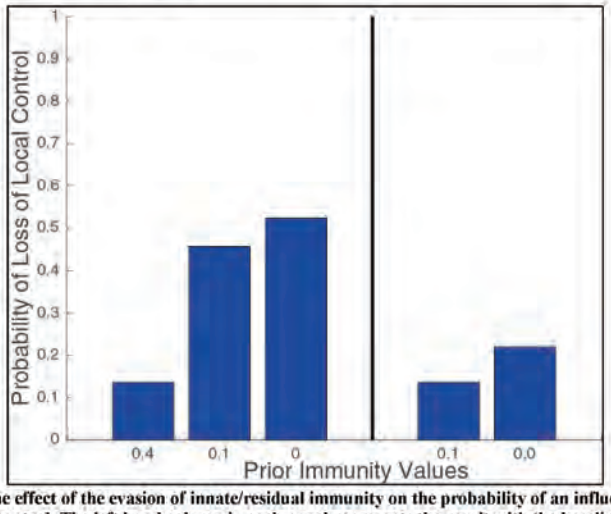

Figure 6.35. The effect of the evasion of innate/residual immunity on the probability of an influenza outbreak escaping local control. The left hand column in each panel represents the result with the baseline value of prior immunity. In the left-hand panel, the presumption is that 40% of the population is protected against infection with a wild type strain of influenza (either seasonal or pandemic, like 1918 H1N1 pdm). Under this condition the effect on the probability of escape if a strain (with the same R_0 value) were able to overcome most of this residual/innate immunity (so that only 10% of the population were immune) or overcome all immunity is shown. In the right-hand panel, the presumption is that 10% of the population has immunity to the wild type strain. Under this condition, the effect on the probability of escape is shown for strains that are modified to overcome all immunity.

This analysis demonstrates that the evasion of pre-existing immunity can significantly increase the probability of an outbreak of influenza escaping local control, by two-to-three-fold, if the population has a high level of residual immunity (as is likely for seasonal influenza since prior vaccination or illness provides some protection against new strains and 1918 H1N1 pdm influenza). Similar to R_0, this parameter influences the probability of an outbreak escaping by enabling the disease to spread more quickly (because each contact is more likely to result in an infection). Pre-existing immunity can protect a significant proportion of the population if the strain released is similar (or identical) to a strain of influenza that recently circulated, which is one reason why this parameter is highly influential. If the population exhibited relatively low levels of prior immunity, then evasion of prior immunity has little influence on consequences (increasing the probability of escape by less than a fifth).

6.6.3.2 Avian Influenza

Because very few humans have been previously exposed to avian subtypes of influenza and because wild type strains are poorly transmissible in people, residual immunity has essentially no bearing on the probability that an outbreak would escape local control.

6.6.3.3 Coronaviruses

Because very few humans have been previously exposed to SARS- or MERS-CoV, residual immunity has essentially no bearing on the probability that an outbreak would escape local control.

6.6.4 Effect of Loss of Containment Pathways on Risk of Loss of Local Control of an Outbreak

The nature of the incidents that could lead to a loss of containment event affect the probability of an outbreak escaping local control in three ways:

1. Incidents can be covert or overt and faster implementation of control measures is possible with overt incidents,
2. Incidents can initially infect a laboratory worker or member of the public, and
3. Incidents can infect a single person or multiple people

In this section we explore how the loss of containment pathway affects probability of an outbreak.

6.6.4.1 Overt Versus Covert Incidents

Some incidents are easy to recognize by the public health and laboratory safety communities as having a very high probability of causing infections outside the laboratory. In the biosecurity assessment, discussed in Chapter 7, the self-announcing events include mass shootings and bombings of the laboratory. In the biosafety assessment, the only event that poses a risk of loss of containment that falls into this category is the earthquake. If an earthquake strikes a laboratory such that obvious physical damage occurs that breaches the containment suites, the response community is likely to adopt measures assuming that the population is at risk of an infection and potential outbreak. Moreover, the community, fearful of the work done in the laboratory, are likely to significantly change their behaviors. Lastly, many laboratory buildings that house work on wild type influenza- or coronaviruses also house work on other human pathogens, so any work done on influenza- and coronaviruses may contribute only a portion of the overall risk of such an event.

If we assume that control measures can be immediately implemented but these control measures are no stronger than those implemented in a laboratory-based outbreak caused by other events, the probability than an outbreak escapes local control is decreased only modestly if at all (Figure 6.36).

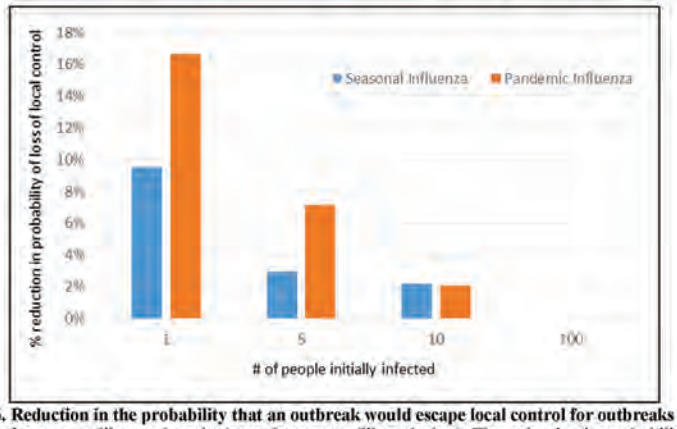

Figure 6.36. Reduction in the probability that an outbreak would escape local control for outbreaks caused by self-announcing events (like earthquakes) vs other events (like splashes). The reduction in probability is small and drops to zero for self-announcing events that initially infect large numbers of people.

If immediate and strong social distancing measures can be adopted (such that people halve the number of contacts they normally have) when an obvious breach in the laboratory is recognized, then no outbreak escapes local control. This result may be intuitively obvious because most outbreaks caused by a wild type influenza virus have an R_0 value less than two and this degree of control would drop the R_0 below one, which is required for the outbreak to be self-sustaining. We have no data to determine how people would behave after a large earthquake destroys a containment laboratory in the context of the chaos caused by the larger event. Perhaps a catastrophic earthquake would naturally reduce the contact between people in the community because school and work will be suspended. Alternatively, perhaps large number of people gathering in shelters would *increase* the contact between individuals and make outbreak control extremely difficult.

Due to the irreducible uncertainty and the minimal effect of the implementation of immediate control measures (which are assumed to be similar in strength to those implemented after a covert loss of containment event), the biosafety analysis assumes that an outbreak in the aftermath of an earthquake that destroys the laboratory has the same chance of coming under local control as any other outbreak. Similarly, in the biosecurity section, since the self-announcing events strike the laboratory with minimal consequences elsewhere (like a bombing or mass shooting), we presume that immediate control measures can be implemented to control the resulting outbreak although these have a minimal additional influence on the outbreak escaping local control.

6.6.4.2 Initial Infections of the Public Versus Initial Infections of Laboratory Workers

If a worker violates the protocol and mingles with the general population while sick, this person has the nearly the same probability of causing an outbreak that spreads beyond local control as an infected member of the public (not shown). The fact that a laboratory worker is trained to report early symptoms

of unusual illness, preemptively self-isolate (and potentially receive prophylactic antivirals) significantly reduces the probability that a worker will not mingle with the general population, as explained above.

6.6.4.3 Initial Numbers of People Infected

Depending on the loss of containment pathway, one, two, or more people could be infected by the event. The vast majority of loss of containment events lead to the infection of one laboratorian who contaminated her hands, failed to decontaminate them thoroughly, and then infected herself and no one else due to the contamination. However, some loss of containment events lead to multiple people infected either directly (via aerosols generated inside the laboratory) or indirectly (via a contaminated worker who happens to physically contact several people soon after leaving the laboratory). Figure 6.37 shows how the probability of an outbreak escaping local control depends on the initial number of people infected for seasonal influenza, pandemic influenza, and SARS. If one person is infected with influenza and mingles with the local population, the outbreak has a 20-30% chance of seeding a global pandemic. As more people are initially infected, the outbreak has a much greater chance of growing beyond local control. Even with 100 initially infected individuals, a SARS outbreak has a minimal chance of escaping local control (unless the R_0 for the pathogen is at the high end of all estimates). In addition, a SARS outbreak has a minimal chance of seeding a global pandemic due to the efficacy of control measures at preventing its spread (unless it is the R_0 for an outbreak in the US is at the high end of estimates of R_0s estimated for this pathogen).

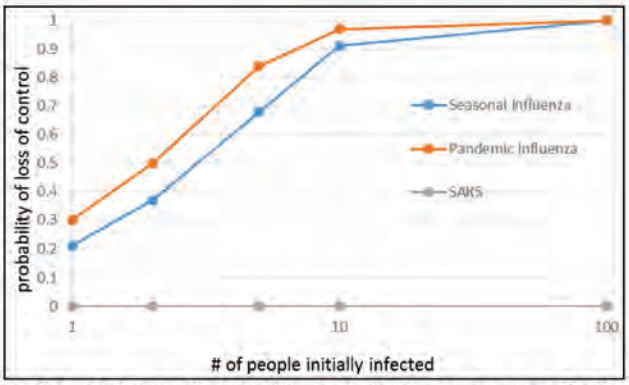

Figure 6.37. The relationship between the probability of an outbreak expanding beyond local control and the number of people initially infected by the loss of containment event. In this figure the median probability of an outbreak not extinguishing across all parameters for seasonal influenza, pandemic influenza and SARS are shown. The X-axis is on a log scale (the data points are for 1, 2, 5, 10 and 100 initial infections).

As explained above, the probability that a loss of containment event leads to the initial infection of one person is much more than ten times as likely as an event that initially infected multiple people. Since the probability of an outbreak escaping local control is not an order of magnitude greater for outbreaks in which more than one person is initially infected, incidents that infect exactly one person dominate the risk of a global outbreak. That is, because incidents that create exactly one index infection happen much more frequently than incidents that create multiple index infections, yet are still relatively likely to cause a global outbreak, these incidents are responsible for most of the global pandemics modeled.

6.7 Consequences of a Global Pandemic of Pathogens that Are Transmissible in Mammals

This section provides a description of the effect of GoF experiments on the consequences of a global pandemic. Because the relative risk of changing any phenotype depends upon the type of pathogen being modified (and its wild type traits), the phenotypes that have the most influence on risk for each pathogen are summarized first. In the sections that follow, a description is provided on exactly how risk changes as those phenotypes are altered.

After an outbreak is initiated, the GoF phenotype of enhanced growth characteristics in culture or eggs no longer has any influence on risk. If this phenotype also increases the transmissibility or pathogenicity in humans, then the models capture changes in risk through those parameters. Moreover, regardless of how the outbreak began, once it has spread globally the consequences of the global pandemic depend on the characteristics of the pathogen, not the means by which the outbreak was initiated.

6.7.1 Seasonal Influenza Virus

Even if a wild type strain of seasonal influenza sparked a global outbreak the consequences of this pandemic would eclipse those from all industrial accidents ever suffered. This section describes which GoF phenotypes would influence the consequences of a global outbreak of seasonal influenza strains. The GoF phenotypes relevant to an ongoing global outbreak of seasonal influenza are:

- The ability to overcome protective vaccination,
- The ability to overcome prior immunity (either natural or induced by previous vaccinations or infection by similar strains in the past),
- Resistance to antivirals,
- Transmissibility, and
- Pathogenicity (used here, case fatality rate)

The ability to evade diagnostics is of secondary importance to the effect of antivirals because diagnostics are used primarily to direct limiting stocks of these antivirals to only those truly infected. Few other effects of evading diagnostics exist at this stage of the outbreak because the agent causing the outbreak would already be identified by the time the outbreak has spread globally and mass vaccination (as soon as a protective vaccine were available) would occur instead of vaccination based on identified cases. Moreover, public health resources are insufficient for case isolation and quarantine when an outbreak has become global.

The GoF phenotypes of enhanced growth is irrelevant to an ongoing outbreak unless it influences transmissibility or pathogenicity (the risk of which are analyzed here). Adaptation to mammalian hosts is irrelevant for this pathogen because it is already adapted to infect and spread amongst humans.

To understand how various GoF phenotypes influence the consequence of a global outbreak, a sensitivity analysis was performed using the BARDA Interactive Influenza Model. In Figure 6.38 below, the value of any given parameter was set at its lowest level or its highest level to produce the mean numbers of deaths globally for those two values across model runs for all values of all other parameters. All parameters can be explored with this analysis simultaneously EXCEPT for prior/natural immunity in the

population because the estimated R_0 of a disease is calculated given natural levels of immunity (that is, the value of these two parameters are linked). We explore the ability of a pathogen to evade natural immunity separately.

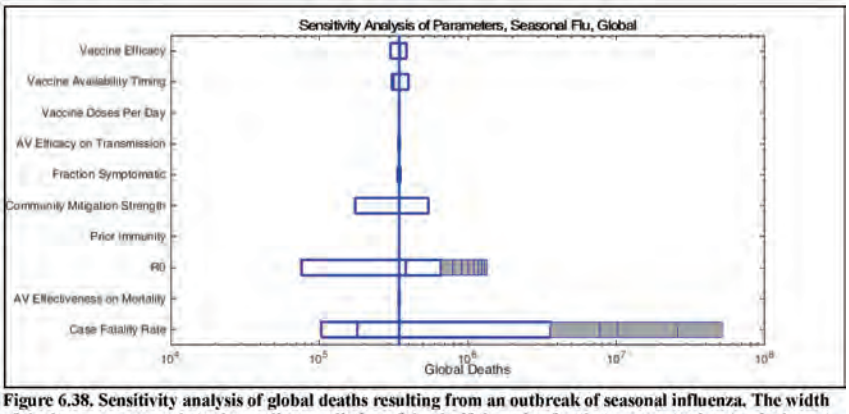

Figure 6.38. Sensitivity analysis of global deaths resulting from an outbreak of seasonal influenza. The width of the boxes corresponds to the median prediction of deaths if the value for that parameter is set to its lowest and highest level (and all other values are allowed to vary). Hollow boxes show the parameter value range for community control measures and wild type pathogens. Grey sections of the boxes show how increases in the transmissibility or pathogenicity beyond wild type levels affect consequences.

Firstly, this analysis demonstrates the great variability possible within the wild type strains that exist. The least deadly (non-attenuated) wild type strains are predicted to cause fifteen-fold fewer deaths globally than the most deadly wild type strains (from 100,000 to four million deaths). The least transmissible wild type strains (that have not circulated recently) are predicted to cause six-fold fewer deaths than the most transmissible wild type strains (from 80,000 to 500,000 deaths). Also, how an outbreak with seasonal influenza would influence global morbidity and mortality in the context of currently circulating strains is unknown. An unresolved question (which likely depends on the biology of the virus released and its similarity to currently circulating strains) is whether the laboratory-associated outbreak would replace the annual toll of seasonal influenza by supplanting circulating strains or would add to this toll. That is, if a laboratory-associated outbreak causes 300,000 deaths, would that be in addition to the several hundred thousand deaths expected annually or replace those expected deaths? Clearly, if a laboratory accident occurred with a wild type, circulating strain, the accident would simply mimic the commonplace occurrence of travel-associated spread of influenza.

This analysis demonstrates that enhancing the pathogenicity of a seasonal influenza strain increases the number of global deaths resulting from an outbreak significantly, largely due to the fact that the case fatality rate of unmodified seasonal influenza is very low. Increasing the case fatality rate from its highest level observed in seasonal influenza to that of 1918 pandemic influenza (5%), increases deaths by more than tenfold.

Increasing the transmissibility of seasonal influenza also increases the number of global deaths significantly, but to a lesser degree than increases in pathogenicity. Increasing the R_0 from 1.4 to 2.2 can double global deaths.

From this analysis, vaccines and antivirals have little influence on the global outbreak because of poor public health infrastructure and resource availability across most of the world. For this reason, the GoF phenotype leading to the evasion of the protection afforded by vaccination or antivirals does not significantly increase global consequences.

However, when the outbreak in North America is considered alone (Figure 6.39), vaccines and antivirals can reduce the deaths by an order of magnitude. This result may be surprising because the outbreak is with an unanticipated serotype so no effective vaccine would be available for months. For a GoF strain of influenza to overcome protection caused by a vaccine made specifically in response to the outbreak this strain is causing, it must be modified to overcome immunity caused by any vaccine, not just a vaccine matched to its serotype. Although the GoF literature describes how to alter the antigenic properties of influenza, no one has described an experiment that makes an influenza strain overcome protective vaccination regardless of its serotype. For this reason, only some GoF experiments leading to the evasion of induced immunity increase consequences.

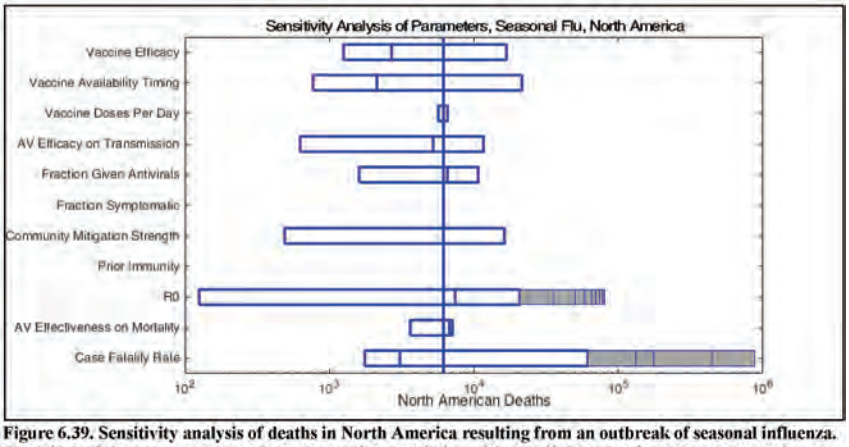

Figure 6.39. Sensitivity analysis of deaths in North America resulting from an outbreak of seasonal influenza. The width of the boxes corresponds to the median prediction of deaths if the value for that parameter is set to its lowest and highest level (and all other values are allowed to vary). Hollow boxes show the parameter value range for community control measures and wild type pathogens. Grey sections of the boxes show how increases in the transmissibility or pathogenicity beyond wild type levels affect consequences.

When considering North America alone, antivirals also can reduce deaths significantly, by about an order of magnitude. Although in a typical seasonal influenza outbreak, only about 5% of patients receive antivirals, federal caches of influenza antivirals could accommodate a much greater level of treatment and so overall death rates could drop significantly (largely through the prevention of secondary infections from those administered antivirals during treatment—compare the width of the bars for AV efficacy at preventing transmission versus AV effectiveness on mortality). For this reason, resistance to antivirals in a modified strain could increase the death toll of an influenza outbreak in the US by about an order of magnitude (fourth and fifth box from top in Figure 6.39) even though this phenotype would have negligible influence on the number of deaths globally (fourth and fifth box from top in Figure 6.38).

6.7.2 Pandemic Influenza Virus

Even if a wild type strain of pandemic influenza sparked a global outbreak, the consequences would eclipse those from all industrial accidents ever suffered. This section describes which GoF phenotypes would influence the consequences of a global outbreak of pandemic influenza strains. The GoF phenotypes relevant to an ongoing global outbreak are:

- The ability to overcome protective vaccination,
- The ability to overcome prior immunity (either natural or induced by previous vaccinations or infection by similar strains in the past),
- Resistance to antivirals,
- Transmissibility, and
- Pathogenicity (used here, case fatality rate).

As above the ability to evade diagnostics is of secondary importance to the effect of antivirals and vaccines and the GoF phenotypes of enhanced growth and adaptation to mammalian hosts are irrelevant to an ongoing outbreak.

To understand how various GoF phenotypes influence the consequence to a global outbreak of pandemic influenza, a sensitivity analysis was performed as described above (Figure 6.40). All parameters can be explored with this analysis simultaneously EXCEPT for prior/natural immunity in the population because the estimated R_0 of a disease is calculated given natural levels of immunity (that is, the value of these two parameters are linked). The ability of a pathogen to evade natural immunity is explored separately.

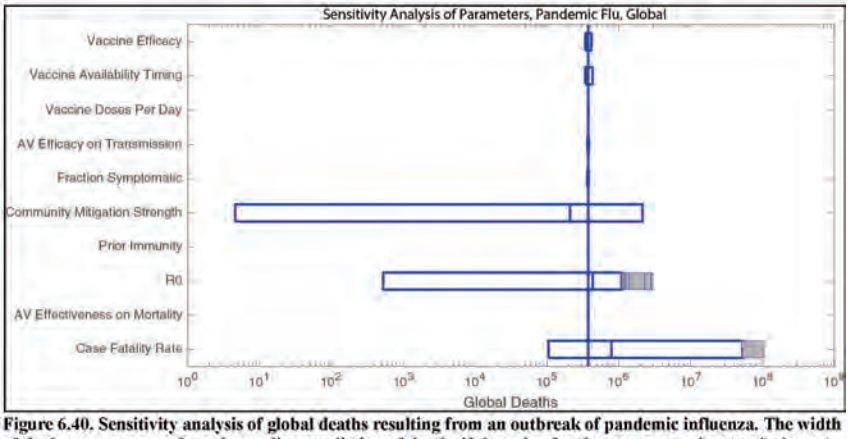

Figure 6.40. Sensitivity analysis of global deaths resulting from an outbreak of pandemic influenza. The width of the boxes corresponds to the median prediction of deaths if the value for that parameter is set to its lowest and highest level (and all other values are allowed to vary). Hollow boxes show the parameter value range for community control measures and wild type pathogens. Grey sections of the boxes show how increases in the transmissibility or pathogenicity beyond wild type levels affect consequences.

Firstly, this analysis demonstrates the great variability possible within the wild type strains that exist. The least deadly (non-attenuated) wild type strains are predicted to cause 400-fold fewer deaths globally than the most deadly wild type strains (from 100,000 to 50 million deaths). This result mirrors our previous observations from the recent 2009 pandemic and the 1918 pandemic, which demonstrated an enormous difference in their case fatality rate. The least transmissible wild type strains are predicted to cause 1,000-fold fewer deaths than the most transmissible wild type strains (from less than a thousand deaths to 1,000,000 deaths).

From this analysis, we note the GoF-related modification of pandemic influenza to increase transmissibility or pathogenicity may influence the global consequences. Vaccines and antivirals have little influence on the global outbreak because of poor public health infrastructure and resource availability across the world. For this reason, the GoF phenotype leading to the evasion of the protection afforded by vaccination or antivirals does not significantly increase global consequences.

When North America is considered alone, for pandemic strains, vaccine evasion and antiviral resistance influences potential deaths about tenfold (Figure 6.41).

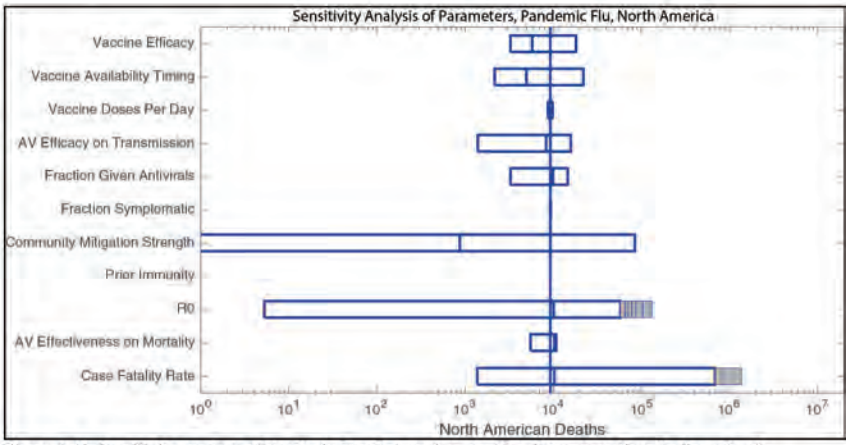

Figure 6.41. Sensitivity analysis of deaths in North America resulting from an outbreak of pandemic influenza. The width of the boxes corresponds to the median prediction of deaths if the value for that parameter is set to its lowest and highest level (and all other values are allowed to vary). Hollow boxes show the parameter value range for community control measures and wild type pathogens. Grey sections of the boxes show how increases in the transmissibility or pathogenicity beyond wild type levels affect consequences.

6.7.3 Avian Influenza Virus That is Transmissible Amongst People

Wild type strains of avian influenza are unable to cause a global pandemic unless they are transmissible amongst people. For this reason, the consequences of a global outbreak for GoF phenotypes other than transmissibility all must be considered in the context of a strain that is already modified to be highly transmissible. This interaction is explored below.

6.7.4 Coronaviruses

Even if a wild type strain of a SARS-like CoV sparked a global outbreak, the consequences would be significant. This section describes which GoF phenotypes would influence the consequences of a global outbreak caused by a SARS-like CoV. We focus on SARS-like CoVs because wild type MERS-CoV is not sufficiently transmissible in people to cause a global pandemic. If MERS-CoV were modified to be more transmissible, the resulting outbreak would resemble that caused by a SARS-like CoV. The GoF phenotypes relevant to an ongoing global outbreak are simply transmissibility and pathogenicity because there are no medical countermeasures to forestall the spread of the illnesses caused by the coronaviruses.

As above, the GoF phenotypes of enhanced growth and adaptation to mammalian hosts are irrelevant to an ongoing outbreak (unless they alter transmissibility or pathogenicity). To understand how various GoF phenotypes influence the consequence to a global outbreak of a SARS-like disease, a sensitivity analysis was performed as described above (Figure 6.42). As the data show, increasing transmissibility of SARS-CoV beyond wild type levels (R_0 of 1.6) can increase median global deaths predicted by several fold, a similar effect to increasing the pathogenicity. If the R_0 value of wild-type SARS-CoV is considered to be 3.0, further increases are of little consequence. Of note, variation in the estimates of wild type transmissibility of SARS-CoV can increase or decrease global deaths by 100,000-fold, showing how little effect a modification can have compared to natural variation (or imperfect epidemiological estimates). Similarly, the ability of the community to reduce their contacts for a significant period of time has a similar influence on the consequences of the outbreak. If the worst-case estimates for transmissibility for a SARS outbreak were used one could expect a global outbreak to kill tens of millions of people. Recall that since SARS is very susceptible to control measures, much of the difference in these estimates is likely due to the robustness of public health measures undertaken to curtail its spread.

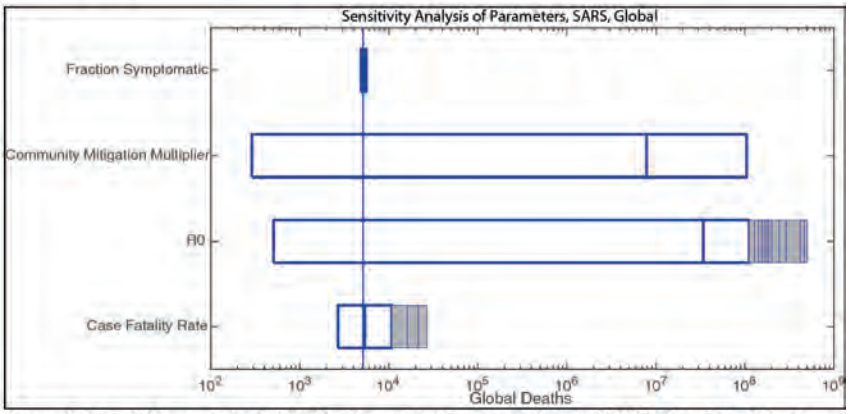

Figure 6.42 Sensitivity analysis of global deaths resulting from an outbreak of SARS. The width of the boxes corresponds to the median prediction of deaths if the value for that parameter is set to its lowest and highest level (and all other values are allowed to vary). Hollow boxes show the parameter value range for community control measures and wild type pathogens (R0 of no greater than 1.6). Grey sections of the boxes show how increases in the transmissibility or pathogenicity beyond wild type levels affect consequences.

Firstly, this analysis demonstrates the variability possible within the wild type strains that exist. The least deadly wild type strains are predicted to cause four-fold fewer deaths globally than the most deadly wild type strains (from 3,000 to 10,000).

A global outbreak of a SARS-like disease would differ from a global influenza outbreak in many ways, several of which are explicitly explored in this analysis (like case fatality rate, existence of medical countermeasures, etc.). Beyond these traits, SARS has a much longer incubation time (median of more than four days but a much greater average) than influenza and therefore a global outbreak of SARS would be much more protracted than an outbreak of influenza. Figure 6.43 shows when the peak number of daily cases of a SARS-like disease is reached compared to the initiation of the outbreak. For the smallest outbreaks, the peak is reached within the first 500 days. However, other outbreaks require many years to reach their peak in terms of cases per day. Clearly, the protracted nature of a SARS-like disease pandemic could put a greater strain on sustaining a response and, conversely, afford some additional opportunities for outbreak control compared to an influenza pandemic that circulates in less than a year. Given that an outbreak of this kind has never been experienced, the nature and effect of these possibilities cannot be quantified in the current modeling effort.

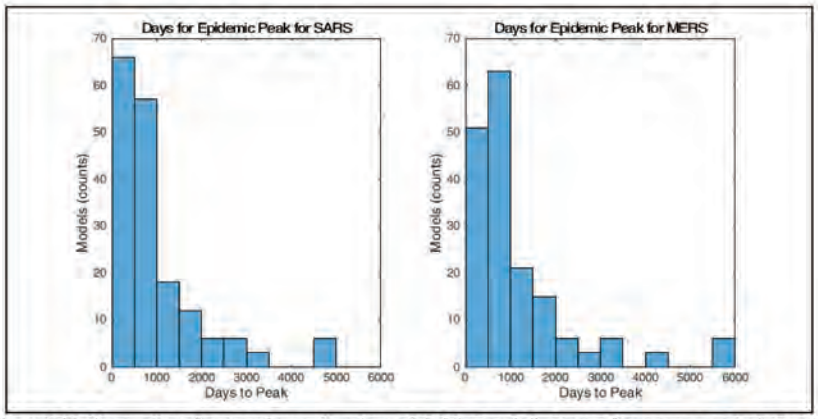

Figure 6.43. The number of coronavirus outbreaks modeled that peak (in terms of new cases per day) at any particular day after the global outbreak begins. To show the duration of truly global outbreaks, outbreaks that lead to less than one million infections are not shown.

The sections that follow provide a drill-down to describe HOW changes in any of the GoF phenotypes affect the consequences of a global outbreak.

6.7.5 Effect of Enhanced Transmissibility in Mammals on Consequences of a Global Outbreak Seasonal influenza

As discussed above, increasing the transmissibility of a seasonal influenza strain can double the global death toll. Figure 6.44 explores this relationship in more detail. These data show that increasing the transmissibility of seasonal influenza to match that of an average pandemic influenza outbreak (R_0 of 1.7) is sufficient to double the death toll and increases beyond that point do no further increase consequences significantly. For relatively poorly transmissible strains of seasonal influenza, increasing the transmissibility to the greatest levels observed for wild type strains (in blue on the left in Figure 6.44) can

increase global deaths by 50 fold if no social distancing measures are taken during the outbreak. Recall that community mitigation is a parameter that describes the actions taken by the public to reduce their contacts with potentially infected individuals (such as avoiding public gatherings and mass transit). Essentially, community mitigation reduces the ability of the disease to spread effectively in the population.

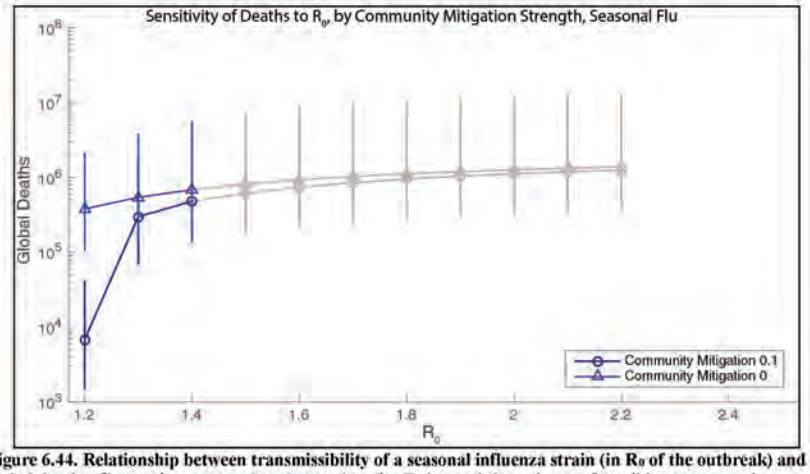

Figure 6.44. Relationship between transmissibility of a seasonal influenza strain (in R_0 of the outbreak) and global deaths. Grey points are used to show values for R_0 beyond the estimates for wild type seasonal influenza strains corresponding to the tornado plot in Figure 6.27. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.5.1 Pandemic Influenza

Increasing the transmissibility of a pandemic influenza strain can increase global risk if the wild type strain is poorly transmissible (such as 1918 H1N1 pdm due to the protection afforded by recently circulating strains). In contrast, if the strain is highly transmissible (like H2 strains) further increases in transmissibility are not significant. For strains with a R_0 of 1.2 (such as 1918 H1N1 pdm in today's population), any increase in transmissibility can increase global consequences by at least 100-fold if any community mitigation but the most stringent is assumed (even a reduction in contacts by 10%--community mitigation of 0.1). In contrast, for the most transmissible strains, increasing transmissibility increases global consequences only if the most severe community mitigation is assumed (a sustained reduction of contacts by 50%). Given that these outbreaks last many months, the ability for the community to sustain this level of social distancing is doubtful, especially given that this level of community mitigation has not been observed in any prior modern influenza outbreak. Figure 6.45 explores this relationship in more detail

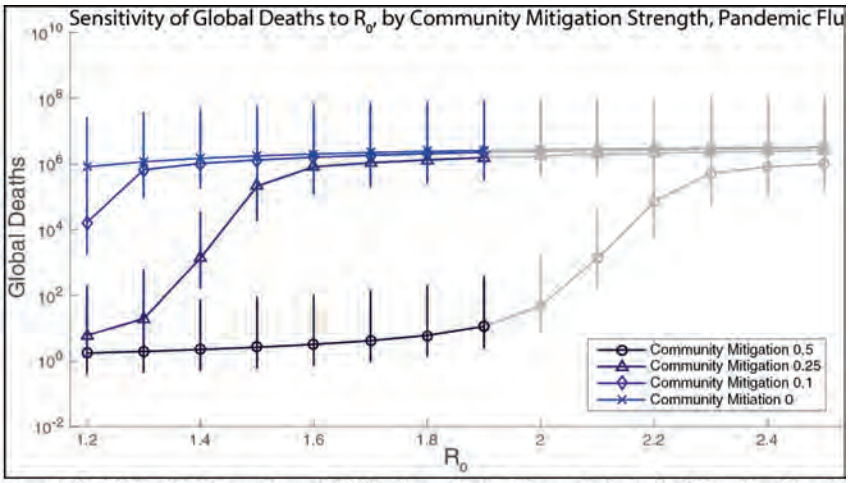

Figure 6.45. Relationship between transmissibility of a pandemic influenza strain (in R_0 of the outbreak) and global deaths for various levels of sustained community mitigation. Grey point are used to show values for R_0 beyond the estimates for wild type pandemic influenza strains and correspond to colors in Figure 6.29. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.5.2 Avian Influenza

In Figure 6.46, we show the relationship between R_0 and global cases of avian influenza for four levels of community mitigation. With a novel, highly pathogenic illness, we can expect the public to significantly change their behavior, as was observed in the SARS outbreak in Canada. However, we lack the data to predict to what degree social distancing can be implemented and for what period of time. Figure 6.46 shows, however, that unless very significant levels of community mitigation can be sustained for a very long time, the number of global cases significantly increases as the R_0 of an avian influenza strain approaches that of seasonal influenza. Increasing the R_0 past 1.5 (which is typical for pandemic influenza strains) has no further effect on consequences. If, however, community mitigation can be sustained at a very high level, then to significantly increase global consequences, the avian influenza strain must be more transmissible than any pandemic influenza strain ever observed. Because the ability of the community to reduce their contacts for a significant period of time is dubious, we presume that the increase of the transmissibility of avian influenza to that of seasonal influenza significantly drives the potential consequences of an outbreak.

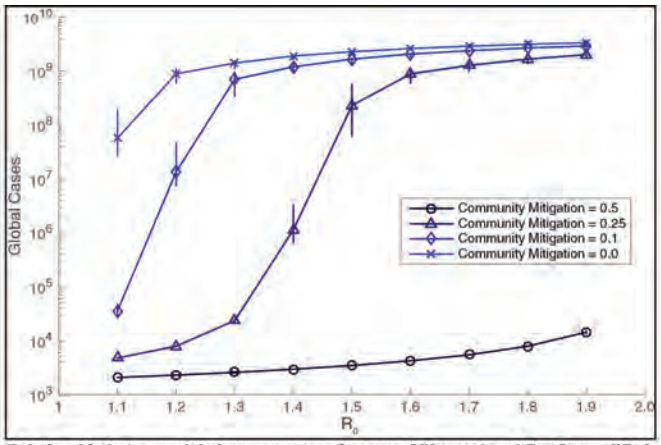

Figure 6.46. Relationship between global consequences (in term of illnesses) and R_0 of a modified avian influenza virus and the strength of community mitigation. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.5.3 Coronaviruses

Figure 6.47 shows the relationship between R_0 and global cases of a SARS-like CoV for three levels of community mitigation. The data show that for wild type strains of SARS-like CoV, the virus is already sufficiently transmissible ($R_0 > 1.4$) to maximize global deaths unless very significant levels of community mitigation can be sustained for a very long time. Figure 6.47 shows results for a SARS-like CoV, but the results for a MERS-like CoV are nearly the same (not shown). That being said, because MERS-CoV is less transmissible than SARS-CoV, a greater increase in transmissibility over a wild type strain is required to produce the same increase in risk. Because the ability of the community to reduce their contacts for the years required for a global outbreak to run its course is unknown, these data suggest that SARS-like CoVs are already sufficiently transmissible to maximize a global outbreak and modifications that increase transmissibility are of little additional risk. Notably, if wild type SARS-CoV already is extremely transmissible ($R_0 > 2.0$) as some have suggested, then even sustained and robust community mitigation will not limit the outbreak. If this were the case, further increases in transmissibility would not increase risk.

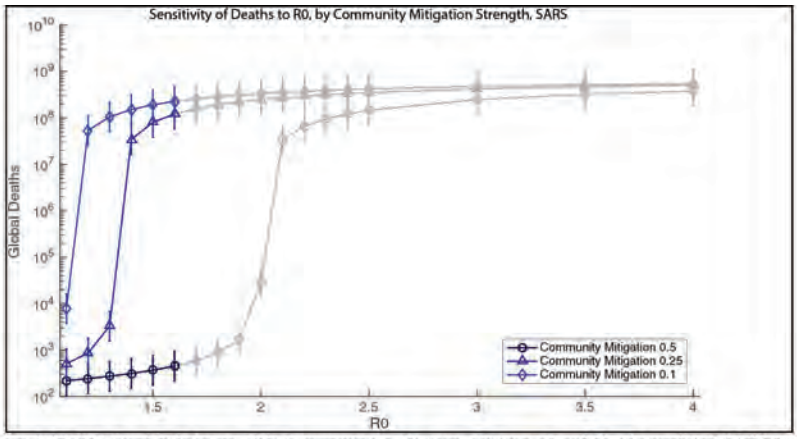

Figure 6.47. Relationship between global consequences (in term of illnesses) and R_0 of a modified SARS-like CoV and the strength of community mitigation. Strains modified to increase transmissibility beyond estimates for wild type SARS-CoV (here 1.6) are shown in grey. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.6 Effect of Enhanced Pathogenicity on Consequences of a Global Outbreak

6.7.6.1 Seasonal Influenza

Because of the low case fatality rate of typical seasonal influenza strains, increasing the pathogenicity of these strains can significantly increase the predicted global death toll (Figure 6.48). These data show that, as expected, increasing the case fatality rate by a factor of 10 or 100 increases the global deaths correspondingly. An increase of this magnitude would be reflected by the modification of a typical seasonal influenza strain to have the pathogenicity of the 1918 pandemic strain.

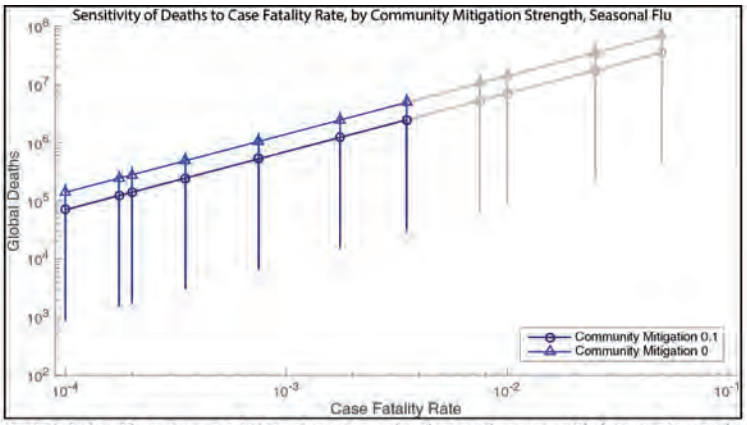

Figure 6.48. Relationship between global deaths and case fatality rate for seasonal influenza. Grey points are used to show values for R_0 beyond the estimates for wild type seasonal influenza strains corresponding to the tornado plot in Figure 6.27. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.6.2 Pandemic Influenza

Because the wild type 1918 pandemic influenza strain had a high case fatality rate, increasing this rate by a factor of 100 is impossible. Figure 6.49 shows the effect on global deaths of doubling the case fatality rate of a pandemic strain to be 10% (double that of the wild type 1918 strain). These data show that, as expected, doubling the case fatality rate doubles the global deaths correspondingly. Death rates beyond 10% have been observed in avian influenza strains only and then only rarely.

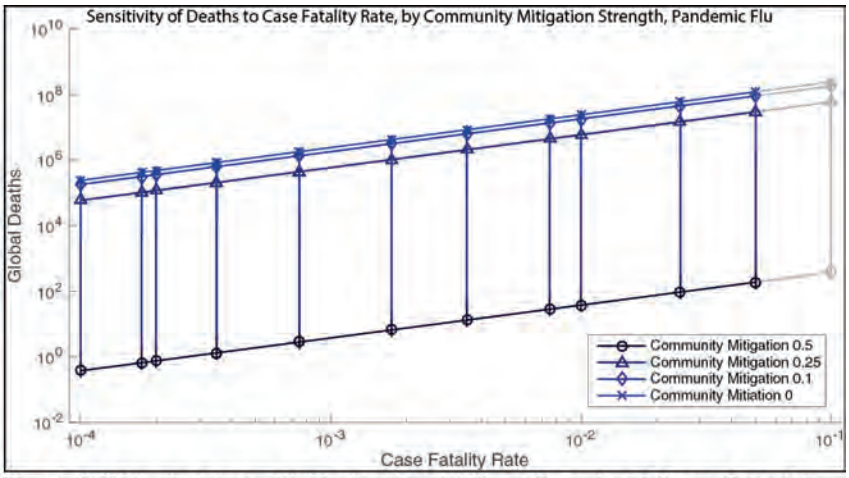

Figure 6.49. Relationship between global deaths and case fatality rate for pandemic influenza. Grey points are used to show values for R_0 beyond the estimates for wild type seasonal influenza strains corresponding to the tornado plot in Figure 6.27. Because the wild type 1918 pandemic strain has a case fatality rate of 5%, much of this graph is occupied by data reflecting wild type strains. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.6.3 Avian Influenza

If a strain of avian influenza that is already modified to be highly transmissible in people was further modified to be more pathogenic in people, the number of deaths is expected to increase. The natural range of pathogenicity in wild type strains of avian influenza is immense, from those that produce no clinical symptoms in humans to those that kill about half of those with recognized illness. For this reason, the GoF study that increases risk most is one in which the already pathogenic strain is made more transmissible while pathogenicity is maintained, not a GoF study in which pathogenicity is increased. However, as shown in Figure 6.50, below, expected global deaths increase linearly with increases in pathogenicity. Figure 6.50 also confirms how much an influence contagiousness has on consequences, as increasing the transmissibility beyond an R_0 of 1.1 increases deaths by at least 10,000 fold.

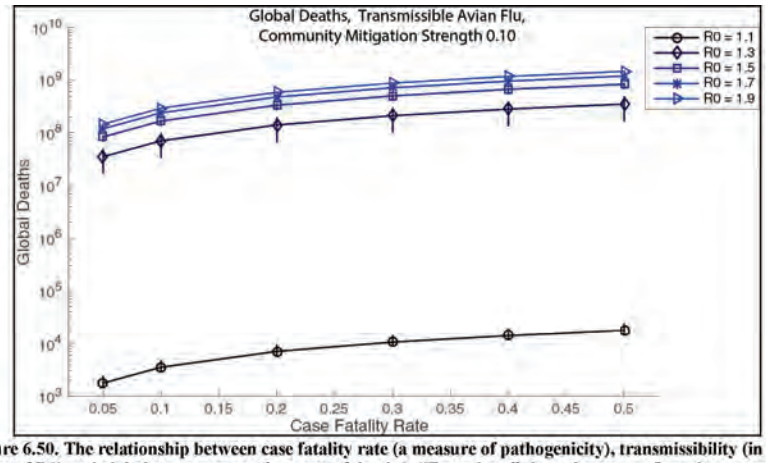

Figure 6.50. The relationship between case fatality rate (a measure of pathogenicity), transmissibility (in terms of R0) and global consequences (in terms of deaths). "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.6.4 Coronaviruses

Modifications to increase the pathogenicity (in this case, the case fatality rate) of a SARS-like CoV have the expected outcome in terms of global deaths as shown in Figure 6.51, in that doubling or tripling the rate of death doubles or triples global deaths.

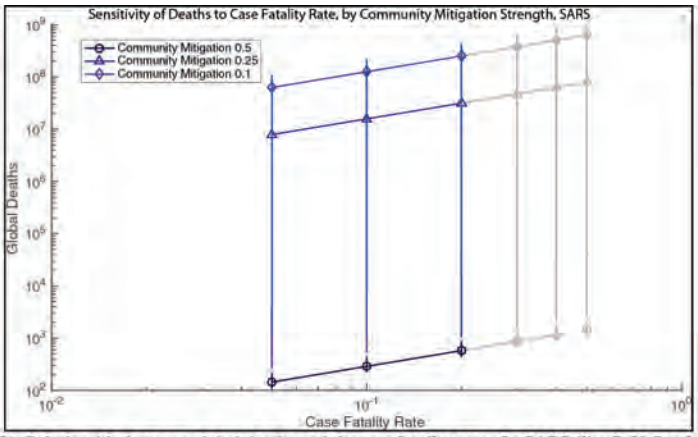

Figure 6.51. Relationship between global deaths and the case fatality rate of a SARS-like CoV. Strains modified to increase pathogenicity are shown in grey. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.7 Effect of Countermeasures Evasion on Consequences of a Global Outbreak

6.7.7.1 Seasonal Influenza

If the strain involved in the laboratory released were able to overcome protective vaccination, the outbreak in North America would cause up to four-fold more deaths (Figure 6.52—vaccine efficacy dropping from 0.6 or 0.4 to 0). Recall that the state of the public health infrastructure in the majority of the world is so parlous that vaccines do not appreciably affect global death rates for influenza, so this risk is realized only by high income countries. Also, this risk is realized only if the outbreak is caused by a strain of influenza that can overcome protective immunity afforded by any vaccine (instead of simply changing the antigenic properties of the virus) because even if a novel strain has unprecedented antigenic properties, a vaccine developed in the midst of an outbreak would be raised to the strain causing the nascent outbreak. For these reasons, this GoF trait poses little overall biosafety risk.

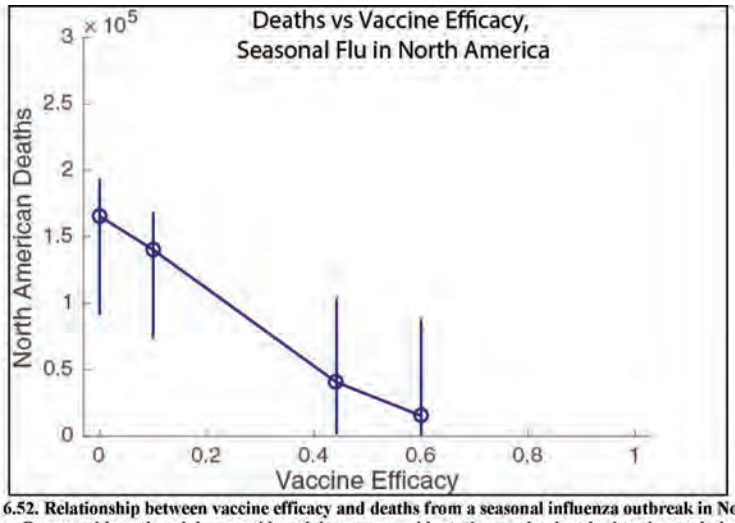

Figure 6.52. Relationship between vaccine efficacy and deaths from a seasonal influenza outbreak in North America. Because this outbreak is caused by a laboratory accident, the vaccine is raised to the strain involved in the outbreak soon after the outbreak occurs. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

In a typical influenza seasons, only approximately five percent of patients receive antivirals. If this level of antiviral distribution is used in the face of an outbreak caused by a laboratory accident, very few lives are saved by antivirals and therefore antiviral resistance has limited influence on risk (Figure 6.53—darker green line). However, the US holds a very large federal cache of antivirals that could be used to provide treatment for many victims in a serious influenza epidemic. One may presume that if a global outbreak were caused by an accident in a US laboratory, this cache would be deployed and used aggressively. In this case, antivirals can significantly reduce risk of an outbreak by preventing the onward transmission of influenza (Figure 6.53—light green line). Conversely, a seasonal influenza strain that is antiviral resistant could vitiate the protection afforded to the public by antivirals and could increase the consequences of an outbreak in North America by five-fold. We do not know how many other countries have similar large caches of antivirals so we cannot determine if this risk increase would be shared by the rest of the high income countries.

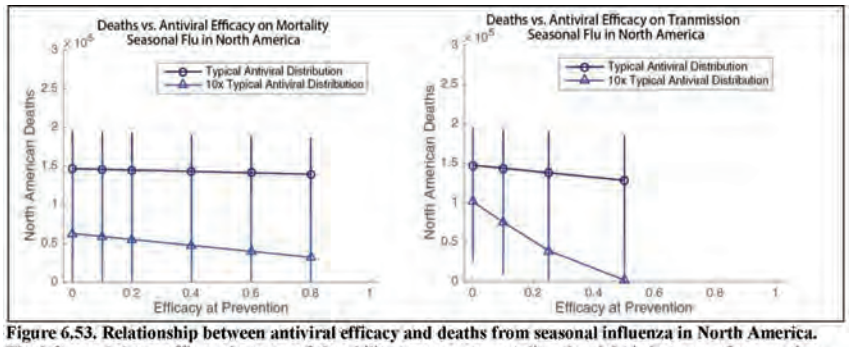

Figure 6.53. Relationship between antiviral efficacy and deaths from seasonal influenza in North America. The left panel shows efficacy in terms of the ability to prevent mortality, the right is in terms of preventing onward transmission. Typically, only about 5% of influenza patients receive antivirals. However, the US has a large cache of antivirals that could be used in case of an emergency. Typical use and possible use are shown in the graph. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.7.2 Pandemic Influenza

If the strain involved in the laboratory released were able to overcome protective vaccination, the outbreak in North America would cause up to four-fold more deaths (Figure 6.54—vaccine efficacy, defined by the percent reduction in infection risk for a vaccinated individual compared to an unvaccinated individual, dropping from 0.6 or 0.4 to 0). Recall that the state of the public health infrastructure in the majority of the world is so parlous that vaccines do not appreciably affect global death rates for influenza, so this risk is realized only by high income countries. Also, this risk is realized only if the outbreak is caused by a strain of influenza that can overcome protective immunity afforded by any vaccine (instead of simply changing the antigenic properties of the virus) because the vaccine would be raised to the strain causing the nascent outbreak. For these reasons, this GoF trait poses little overall biosafety risk.

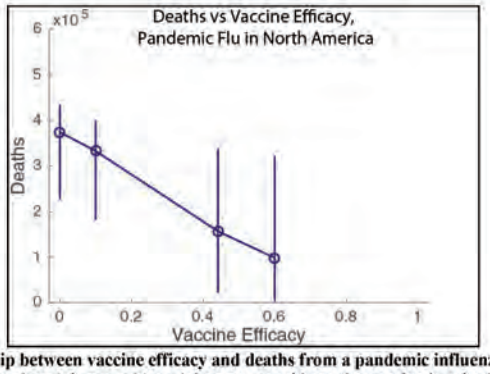

Figure 6.54. Relationship between vaccine efficacy and deaths from a pandemic influenza outbreak in North America. Because this outbreak is caused by a laboratory accident, the vaccine is raised to the strain involved in the outbreak soon after the outbreak occurs. "Error bars" show the range of results across 80% of the

parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

If only 5% of the population receives antivirals, then risk is barely mitigated (Figure 6.55—darker blue line). If antivirals were more widely distributed, these countermeasures can reduce risk of an outbreak by two- to four-fold by preventing the onward transmission of influenza and by preventing mortality (Figure 6.55—light blue line). Conversely, a pandemic influenza strain that is antiviral resistant could vitiate the protection afforded to the public by antivirals and could increase the consequences of an outbreak in North America by two-to four-fold. We do not know how many other countries have similar large caches of antivirals so we cannot determine if this risk increase would be shared by the rest of the high income countries.

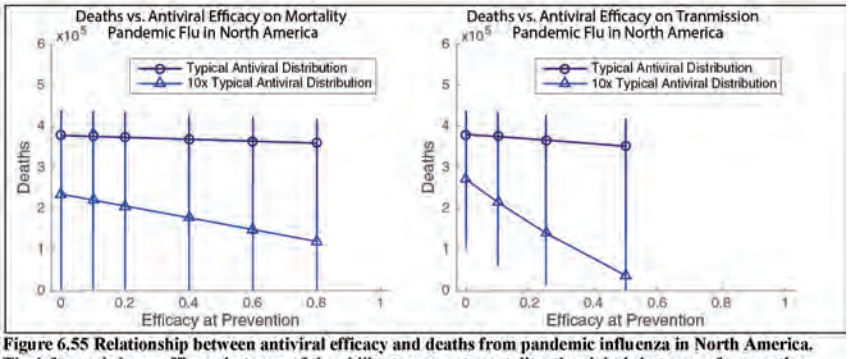

Figure 6.55 Relationship between antiviral efficacy and deaths from pandemic influenza in North America. The left panel shows efficacy in terms of the ability to prevent mortality, the right is in terms of preventing onward transmission. Typical use and possible use are shown in the graph. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented).

6.7.7.3 Avian Influenza

An unexpected outbreak of avian influenza that is highly transmissible amongst people would be very difficult to control with vaccination. The disease will have spread significantly by the time a protective vaccine could be developed, tested, made in quantity and deployed. For this reason, as shown in Figure 6.56, below, the efficacy of the vaccine (and therefore, the ability of the pathogen to evade protective vaccination), matters only for a narrow range of R_0 values for avian strains. North America is shown because this region has greater resources and capacity than the world as a whole so that vaccines can show some efficacy. If the strain is highly transmissible, then vaccination comes too late to prevent a significant number of deaths. If the strain is as transmissible as the least transmissible seasonal influenza strains, then community mitigation is sufficient to contain the outbreak to a relatively low level without vaccination.

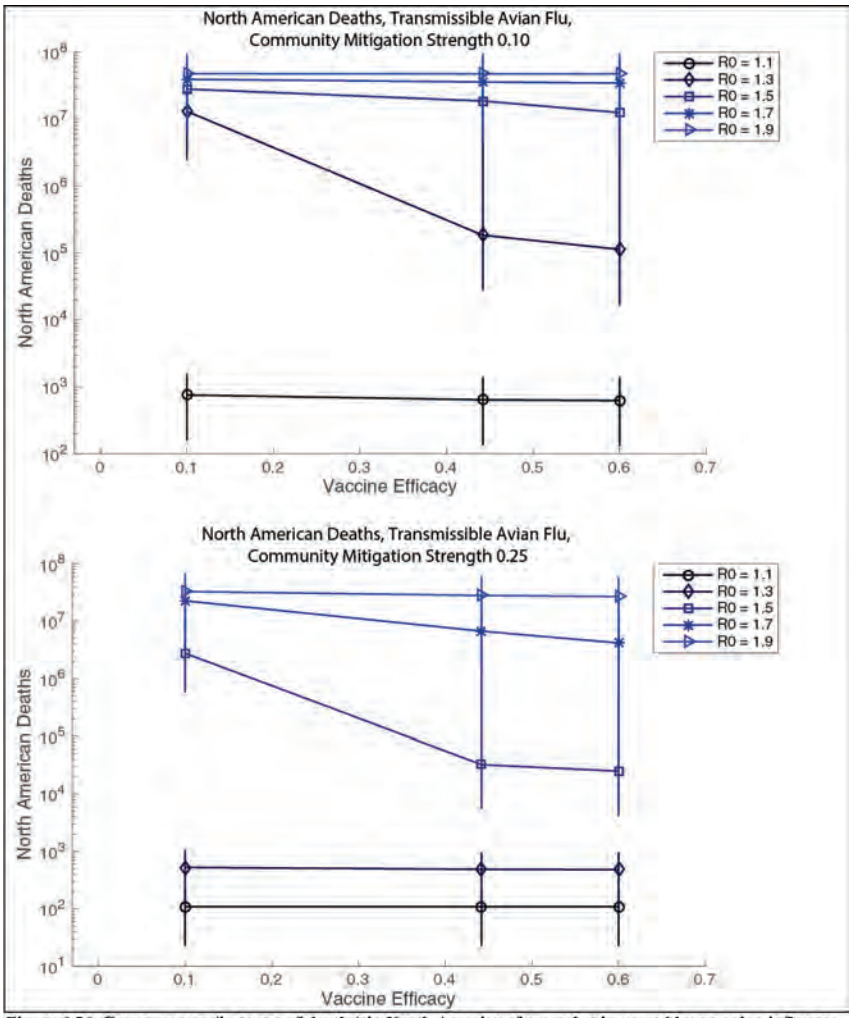

Figure 6.56. Consequences (in terms of deaths) in North America of a pandemic caused by an avian influenza strain modified to be highly transmissible amongst people as a function of vaccine efficacy and transmissibility. "Error bars" show the range of results across 80% of the parameter values explored in the analysis (the parameter values that cause the highest and lowest 10% of results are not represented). The panel above shows a modest level of community mitigation sustained throughout the pandemic, and the panel below shows a more robust level of community mitigation sustained through the pandemic.

Two critical points must be made. Firstly, because a vaccine will be developed for the serotype driving the outbreak, the evasion of a vaccine is only relevant if the strain is modified to evade protection by any vaccine, regardless of the antigenic properties of the virus. Secondly, predicting how transmissible an avian strain could become is difficult. Although seasonal influenza strains are highly transmissible, an avian strain could, theoretically, become as transmissible as a pandemic strain if much of the increase in transmissibility of pandemic strains over seasonal strains is due to the lack of protective innate or residual immunity in the population. For this reason, the value of protective vaccination against an unexpected outbreak caused by an avian strain is very difficult to predict with certainty.

In summary, two facts significantly limit the risk posed by a transmissible avian strain of influenza that can evade vaccination. Firstly, a narrow combination of phenotypes and control measures are necessary for vaccines to have a significant effect on the outbreak even in North America (where the response capacity is much greater than the world as a whole). Secondly, to affect risk at all, the strain must be able to overcome protective vaccination regardless of the serotype of the virus. Although this modification poses a biosecurity risk (see below), it is not the subject of active research (and also of dubious scientific benefits) and so poses little biosafety risk.

6.7.7.4 Coronaviruses

Currently, no countermeasures specific to infections by the coronavirus are used to treat illnesses caused by this pathogen or prevent the ongoing spread of an outbreak caused by this pathogen. For this reason, this phenotype has no influence on risk.

6.7.8 Effect of Evasion of Natural/Residual Immunity on Consequences of a Global Outbreak

6.7.8.1 Seasonal Influenza

As mentioned above, the baseline sensitivity analysis does not investigate the sensitivity to innate/residual immunity in the population, which can be significant for seasonal influenza strains, because population immunity is already accounted for in the effective R_0. In Figure 6.57 below, we investigate how changes in innate or residual immunity affects global deaths.

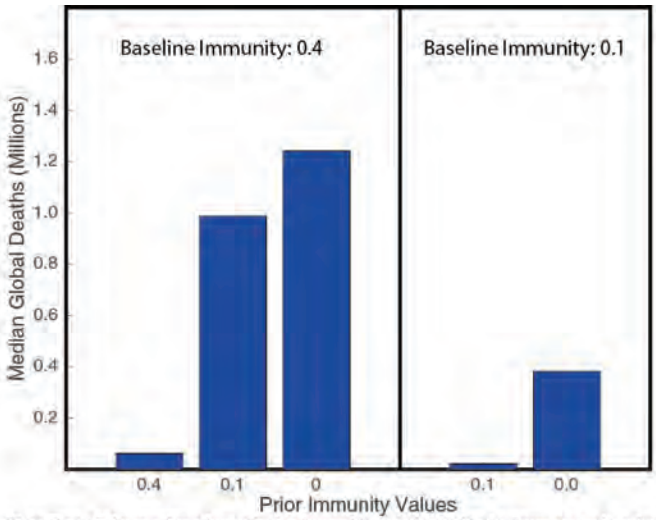

Figure 6.57. The effect of the evasion of population immunity on global deaths from an outbreak of seasonal influenza. The result for the baseline parameter value for prior immunity is the leftmost column in each panel. In the left-hand panel, the baseline assumption is that 40% of the population is protected against infection with a wild type strain. The graph shows the increase in the number of deaths when a strain (with the same R_0 value) were able to overcome most of the immunity (so that only 10% of the population were immune) or overcome all immunity. In the right-hand panel, the baseline assumption is that 10% of the population has immunity to the wild type strain. The graph shows the increase in number of deaths when the strain is modified to overcome all immunity.

This analysis demonstrates that the evasion of pre-existing immunity can increase global deaths by ten-fold, if the population has a high level of residual immunity (as is likely for seasonal influenza since prior vaccination or illness provides some protection against new strains). Similar to R_0, this parameter influences the global outbreak by enabling the disease to spread more quickly (because each contact is more likely to result in an infection) and eventually infect a larger number of people worldwide. Pre-existing immunity can protect a significant proportion of the population if the strain released is similar (or identical) to a strain of influenza that recently circulated, which is one reason why this parameter is influential. If the population exhibited relatively low levels of prior immunity, then evasion of prior immunity has a smaller influence on consequences.

6.7.8.2 Pandemic Influenza

As mentioned above, the baseline sensitivity analysis does not investigate the sensitivity to innate/residual immunity in the population because population immunity is already accounted for in the effective R_0. In Figure 6.58 below, we investigate how changes in innate or residual immunity affects global deaths.

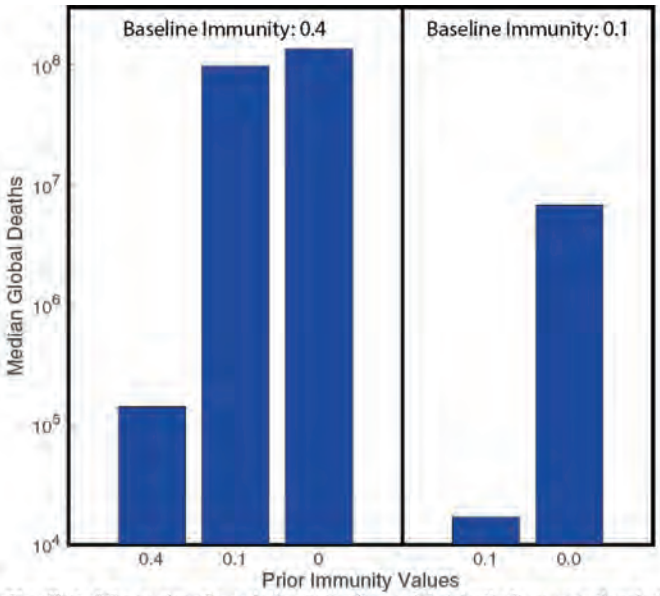

Figure 6.58. The effect of the evasion of population immunity on global deaths from an outbreak of pandemic influenza. The result for the baseline parameter value for prior immunity is the leftmost column in each panel. In the left-hand panel, the baseline assumption is that 40% of the population is protected against infection with a wild type strain. The graph shows the increase in the number of deaths when a strain (with the same R_0 value) were able to overcome most of the immunity (so that only 10% of the population were immune) or overcome all immunity. In the right-hand panel, the baseline assumption is that 10% of the population has immunity to the wild type strain. The graph shows the increase in number of deaths when the strain is modified to overcome all immunity.

This analysis demonstrates that the evasion of pre-existing immunity can greatly increase global consequences for those pandemic strains against which the population has a significant immunity (1918 H1N1 pdm and 2009 H1N1 pdm). In fact, this parameter can increase the expected global deaths from an outbreak of 1918 H1N1 pdm by a factor of 1,000. Even if pre-existing immunity is minimal but non-zero, the expected deaths caused by an outbreak that evades this immunity increases by more than 100-fold.

6.7.8.3 Avian Influenza

Exposure to avian influenza strains is so rare in human populations that very few people have residual immunity to this pathogen. For this reason, evasion of residual immunity has no influence on risk.

6.7.8.4 Coronaviruses

Exposure to a coronavirus is so rare in human populations that very few people have residual immunity to this pathogen. For this reason, evasion of residual immunity has no influence on risk.

6.8 Supporting an Estimate of Absolute Risk

This assessment was designed to evaluate the increase in risk caused by the creation of strains of pathogens with GoF traits compared to wild type pathogens. This approach enabled the assessment to capitalize on the strengths of the available data and minimize the importance of the weaknesses. Sufficient biomedical and epidemiological evidence exists to develop robust models of the initiation of an outbreak from the primary to the secondary cases and the expansion of this outbreak within a community to eventually spark a global pandemic. In contrast, very little data exists on human reliability in life science laboratories, which drives the probability that laboratory acquired infections occur in the first place. Fortunately, the accidents that humans cause (or contribute to) in the laboratory are the same regardless of the pathogen manipulated. That is, workers may overfill a centrifuge tube with the same frequency regardless of the pathogen in the tube or will slip while working with scissors during a necropsy with the same frequency regardless of the pathogen studied. Because the absolute rate at which these accidents happen and cause infections is not supported by robust data, absolute estimates of the rate of laboratory acquired infections cannot be made using the method described in this report.

However, to provide a context for the increase in risk suffered, absolute risk estimates are desired. For this reason, the historical rate of laboratory acquired infections could be used to predict a reasonable upper bound for the frequency with which these incidents occur. However, the research team is unaware of any laboratory acquired infections in laboratories that study influenza or coronaviruses, and so an absolute risk analysis will have at its foundation a weak estimate of the frequency at which laboratory acquired infections occur. That being said, this historical rate of laboratory infections can then be combined with calculated rates of laboratory acquired infections leading to secondary infections, local outbreaks, and global pandemics from this assessment to produce an estimate of absolute risk.

The return frequency of laboratory acquired infections (LAIs) was estimated for several hypothetical historical LAI counts, presuming that some historical LAIs may have gone undetected or unreported. LAI frequency was modeled using a binomial distribution, with the number of trials set to the number of laboratory-years (i.e., the number of laboratories working with the viruses times the observation period), and the number of "successes" equal to the number of LAIs. For influenza, 100 labs[364] and an observation period of twenty years (for a total of 2,000 lab-years) was assumed because there has been roughly 20 years since the expansion of the life science research in the mid-1990s. This time period also coincides with a wave of construction of modern biocontainment facilities that better represent the safety conditions of today's laboratories than previous laboratories.

Shown are the limits of the two-sided 80% confidence interval on the expected LAI frequency, estimated using a Clopper-Pearson interval.[365] The maximum likelihood estimate (MLE) of the frequency with which an LAI would occur (the return frequency) was computed as the number of laboratory years divided by the number of LAIs. Note that, for zero observed LAIs, the minimum and MLE return rate approach infinity and are not plotted.

The project team knows of no laboratory acquired infections involving any one of these laboratories.[366] This lack of a laboratory acquired infection could be due to the fact that none have occurred in that time frame or that some have occurred but the project team does not have access to the reports or data. Figure 6.59 shows the limits of the 90% confidence interval (90 out of 100 times, LAIs would happen, on

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[364] The exact number of laboratories does not significantly influence absolute risk (the per-laboratory rate decreases but the absolute rate of an accident across all laboratories does not change).

[365] Newcombe RG (1998) Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in medicine* 17: 857-872.

[366] At least one parenteral exposure to H5N1 has occurred, and this worker was isolated, but never became ill, probably because influenza is a respiratory pathogen and cannot infect muscle.

average, less often) and maximum likelihood estimate of the return period of laboratory acquired infections given that zero to ten infections have occurred in the past 20 years in the approximately 100 laboratories.

Across all 100 laboratories, a laboratory acquired infection could be expected as frequently as once every 8.5 years (if no infections have occurred in the last 20 years) to as little as every 200 years (if one infection occurred). If the assumption is made that three LAIs have surreptitiously occurred, then an LAI is expected to occur from once every three years to once every 20 years.

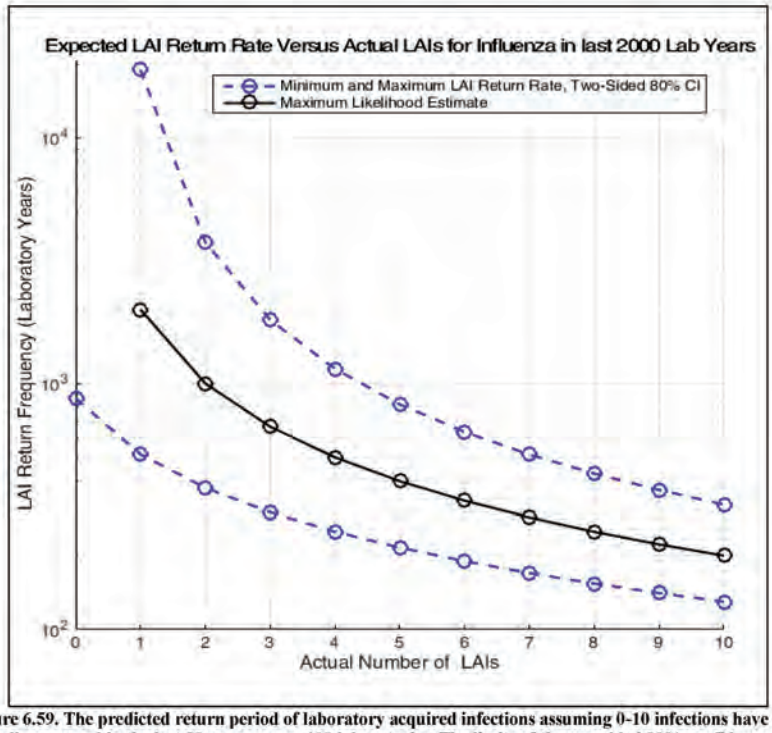

Figure 6.59. The predicted return period of laboratory acquired infections assuming 0-10 infections have actually occurred in the last 20 years across 100 laboratories. The limits of the one-sided 90% confidence interval of the maximum rate (bottom line) was used to produce an estimate of the return period that would be greater than 90 out of 100 actual values of the frequency given the observations, whereas the maximum likelihood estimate and limits of the one-sided 90% confidence interval of the minimum rate (top line) are also shown.

The quantitative analysis in this report estimates that a small minority of these infections would start a local outbreak and a minority of these outbreaks would seed a global pandemic.

For seasonal influenza, the analysis presented above suggests that only 0.4% of LAIs with seasonal influenza are predicted to cause a global pandemic (assuming the strain has not recently circulated, in which case, the probability would be even less). Because most of the 100 laboratories working on the pathogens assessed in this report are studying seasonal influenza, this analysis suggests that a global pandemic would be caused by a laboratory accident in the US once every 2,000-50,000 years (if essentially no LAIs have occurred in influenza laboratories in the past 20 years). If instead the assumption is made that three LAIs have surreptitiously occurred, a global pandemic could be triggered once every 750-5,000 years. It is worthy to note that viruses were characterized much less than 750 years ago, so it cannot be stated with any certainty that these pathogens will be studied under similar containment conditions for long enough into the future for an accident to be likely to occur even once. Moreover, the true consequence of a seasonal influenza outbreak caused by a laboratory accident is unclear. Although predictions can be made about the illnesses and deaths that would be caused, it is unknown how this outbreak would influence the evolution and spread of other influenza strains and if these laboratory-associated infections would supplant or supplement those expected on an annual basis. This caveat aside, the analysis predicts 100,000-4,000,000 deaths to occur from a global outbreak of a wild type seasonal influenza strain, depending on the pathogenicity and transmissibility of the strain.

Considering the other influenza viruses in this study, a historical analysis predicts that LAIs would occur with a similar frequency assuming that no infections have occurred (the per-laboratory rate of LAIs increases but fewer laboratories study these pathogens). This result is obviously counterfactual because these pathogens are manipulated at a greater containment level than wild type, seasonal influenza viruses to decrease the probability of a LAI. That being said, a conservative estimate predicts that laboratory acquired infections occur at the same rate as for seasonal influenza viruses. The analysis presented above suggest that only 1.5% of LAIs with pandemic influenza are predicted to lead to global outbreaks. Combined with the predicted return frequency of LAIs given no LAIs in the last 20 years, a global pandemic caused by research on pandemic influenza viruses is expected every 560-13,000 years. Assuming that no LAIs have occurred with the deadliest pandemic strains is reasonable because, it would be widely known if several laboratory accidents occurred. An accident that sparks an epidemic with a strain as deadly as the 1918 pandemic strain but as transmissible as the 1957 strain could cause up to 80 million global deaths according to the analysis presented above. If, conversely, the accident occurred with a strain similar to the 2009 pandemic strain, it would resemble an accident with seasonal influenza.

Wild type avian influenza strains are not transmissible enough among people to cause a significant local outbreak and therefore no global outbreak is possible. Assuming the same return frequency of laboratory acquired infections for avian influenza as predicted for seasonal influenza, a laboratory worker is expected to fall ill once every three to nine years. For the most pathogenic strains, this worker has a significant chance of dying but the outbreak is likely to extend no further than that one case.

Given that SARS-CoV has been studied for only a decade, the historical record of no laboratory accidents once again suggests that LAIs occur more frequently in coronavirus laboratories at BSL-3 than in laboratories that study seasonal influenza at BSL-2, which is obviously wrong. If, conservatively, the estimate is made that LAIs with SARS-CoV occur as frequently as influenza, a LAI is expected to occur once every 8.5 years (given the seriousness of SARS, LAIs are likely to have been reported so it is safe to assume that no LAIs have occurred yet with this pathogen in the US). The best estimates for the transmissibility of SARS-CoV and its susceptibility to control measures suggest that there is no chance that this outbreak would spark a global pandemic (and SARS-CoV is more transmissible than MERS-CoV). Most of these LAIs would lead to no further infections, however, some would lead to the infection of a handful of other individuals.

6.9 Using the Parametric Risk Assessment: Example Calculation

By design, the biosafety risk assessment is broad and provides data to understand how risk changes if a wild type pathogen is manipulated in one of a variety of ways. This section provides an example illustrating how to simply use the information contained in this report to assess the risk posed by a *particular* manipulation. This example compares the risk of research on two possible modified strains to a wild type strain of influenza (called Strain 1). The example assumes that the strain has not circulated recently so that it itself has some real biosafety risk. This example will use parameter values typical for a wild type seasonal influenza strain as a baseline, which is described by the following parameters:

- Transmissibility: R_0=1.3,
- Pathogenicity: Case fatality rate of 0.001,
- Antiviral sensitivity: (efficacy at preventing transmission=0.25, efficacy at preventing death=0.4),
- Vaccine protection: (efficacy of 0.5 at preventing infection), and
- Infectivity: Set to seasonal influenza (ID_{50} less than 10pfu).

This example uses two modified strains. Strain 2 is a GoF strain of seasonal influenza that is exactly like the wild type strain, except that it is as transmissible as a strain pandemic influenza (R_0=1.7). Strain 3 is an attenuated strain of seasonal influenza that is exactly like the wild type strain, except that it has a case fatality rate of 0.0001.

The modeling completed enables a complete assessment of how any combination of parameter values that describe the pathogen and control measures influences risk, however, all possible combinations of these values and their influence on risk cannot be shown concisely in a report. Instead, static slices through this very complex risk space are taken and shown as two-dimensional figures in this report that explore the effect of changing one parameter while allowing all others to vary. That is, using this report, phenotypes must be assessed individually. The reader will note that the baseline results change regarding which trait is being considered for the same strain (that is, which parameter is held at a particular value while all others are allowed to vary). This phenomenon is expected because the figures show the median and 80th percentile results for all of the parameters that COULD describe a wild type strain and a modified strain (and the same range of values for the control measures). This parameter range will obviously change depending on which trait is held constant.

6.9.1 Step 1: Determine if the Probability of the Pathogen Escaping the Laboratory Changes

To determine how the probability of a pathogen escaping the laboratory changes as a pathogen is modified, refer to Section 6.4.4, specifically 6.4.4.1 for seasonal influenza. Table 6.3 shows how any trait affects this probability. Neither of the traits considered in this example influence the probability that a laboratory incident would lead to escape of the pathogen from the laboratory.

Recall that, although the analysis developed for this study will not permit an estimation of how frequently laboratory accidents lead to laboratory acquired infections that spark a local outbreak, historical rates of accidents suggest that a local outbreak would be sparked by a laboratory acquired infection about once every 500-10,000 years.

6.9.2 Step 2: Determine the Change in the Probability of a Resulting Outbreak Escaping Local Control

To determine the change in the probability that an outbreak, caused by a laboratory accident, would escape local control and seed a global pandemic, refer to Section 6.6. This section demonstrates that of the modifications described in this example, only transmissibility is known to have a significant influence on the probability that an outbreak would escape local control. To determine HOW changes in this trait affect this probability, refer to Section 6.6.1 and Figure 6.29, specifically, for seasonal influenza strains. This figure shows that, should an outbreak resulting from an accident occur, this wild type strain (R_0=1.3) has a baseline chance of escaping local of roughly 21% (12-30% using 80% of the parameter values in the assessment). If the highly transmissible Strain 2 (R_0=1.7) were to cause a local outbreak, the probability that the outbreak would escape local control and seed a global pandemic increase to 44% (27-50%). Because, as described in Section 6.2.9 and Figure 6.12, the range of results in the figures reflect monotonic increases with the same overall shape as the median estimate, one can directly compare the median estimate and the extremes of the range given for any set of results. For this reason, this specific modification is estimated to increase the probability that an outbreak would escape local control by 2.1-fold (1.6-2.3x). If the transmissibility could be increased to rival the most transmissible pandemic influenza strains, the risk of local escape would be further increased.

Table 6.8. Summary of the Influence of Exemplar Modifications to Seasonal Influenza on the Probability That an Outbreak, Caused by a Laboratory Accident, Would Escape Local Control and Seed a Global Pandemic

Strain	1=wild type	2=highly transmissible	3=attenuated
Increase in probability of an outbreak escaping local control	1, defined	2.1x (1.6-2.3x)	1, no change due to modification

6.9.3 Step 3: Determine if the Consequences of a Resulting Pandemic Changes

To determine how the consequences of a global pandemic changes, refer to Section 6.7. Within Section 6.7 refer to the section describing each modified trait of interest to understand how changes in that trait affect this probability. This section demonstrates that both of the modifications described in this example affect the consequences of a global pandemic.

To determine how changes in transmissibility affect consequence, refer to Section 6.7.5.1 and Figure 6.44, specifically, for seasonal influenza strains. This figure shows that, should a global pandemic occur, this wild type strain (R_0=1.3) would lead to roughly 500,000 global deaths (150,000-4,000,000 using 80% of the parameter values in the assessment and assuming no community mitigation occurs). If the highly transmissible Strain 2 (R_0=1.8) were to cause a global pandemic, the deaths suffered would increase to 900,000 (250,000-10,000,000). Because, as described in Section 6.29 and Figure 6.12, the range of results shown in the figures reflect monotonic increases with the same overall shape as the median estimate, one can directly compare the median estimate and the high and low parts of the range given for any set of results. For this reason, this specific modification is estimated to increase the consequences of a global pandemic by 1.8-fold (1.6-2.5x).

To determine how modified pathogenicity affects global consequences should a global pandemic occur, refer to Section 6.7.6 and Figure 6.48, specifically for seasonal influenza. Although this figure visually

displays how case fatality rate affects global deaths, the change in deaths is simple to calculate as a tenfold decrease in the case fatality rate simply leads to a tenfold decrease in global deaths.

Table 6.9. Summary of the Influence of Exemplar Modifications to Seasonal Influenza on the Consequences of a Global Pandemic

Strain	1=wild type	2=highly transmissible	3=attenuated
Increase in global consequences	1, defined	1.8x (1.6-2.5x)	0.1

6.9.4 Putting it Together

Because the risk of a pandemic in this study is the product of the frequency of a laboratory incident sparking a local outbreak, the frequency of an outbreak escaping local control and the consequences of a global pandemic, the total change in risk can be simply understood as the product of the increases in any of these values over the baseline.

The highly transmissible Strain 2 is as likely to escape from a laboratory, but 2.1-fold (1.6-2.3x) more likely to cause a pandemic that would kill 1.8-fold (1.6-2.5x) more people than the wild type strain. In total then, research on this strain poses 3.8-fold (2.6-5.8x) more risk of pandemics than research on a wild type seasonal influenza strain. Put another way, GoF experiments that increase the transmissibility of seasonal influenza to the level of pandemic influenza strains are 3.8-fold more risky than alternate experiments involving wild type strains. In contrast, if the research could be conducted with an attenuated strain instead of a wild type strain, risk would decrease by a further tenfold.

7 Biosecurity Risk of Malicious Acts Targeting a GoF Laboratory

7.1 Biosecurity Risk Assessment: Summary

The purpose of the biosecurity risk assessment is to provide NSABB with an assessment of the likelihood that a malicious act involving a GoF influenza, SARS-CoV, or MERS-CoV virus could result in local infections or widespread pandemic. The risk assessment involved five steps: 1) characterization of the threat, which includes an evaluation of historical incidents and malicious actor motivation and capability (the "offense"); 2) review of the current security policies and practices landscape that governs research with influenza, SARS-CoV, and MERS-CoV in the United States (the "defense"); 3) identification of plausible threats based on analysis of the "offense" and "defense"; 4) assessment of the potential for the plausible threats to cause infections in the local community or broader; and 5) comparison of possible pandemic consequences of plausible threats involving GoF viruses and non-GoF viruses.

No unclassified information describing the threats to research laboratories that store or study GoF influenza, SARS-CoV, or MERS-CoV virus is available. Therefore, to identify the types of actors and acts that may target a GoF laboratory, our approach involved examining historical incidents involving life science laboratories and hospitals, evaluating the motivations and capabilities of malicious actors, and determining if and how existing security measures affect the likelihood of success of a malicious act. Plausible threats facing laboratories that study or store GoF virus(s) were extrapolated from this assessment. Figure 7.1 presents a schematic of the biosecurity risk assessment process.

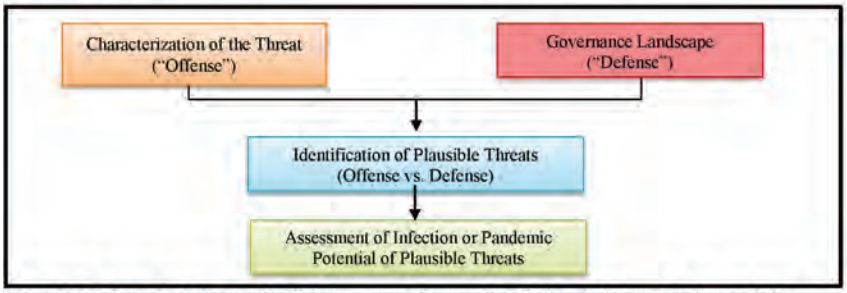

Figure 7.1. Schematic of Biosecurity Risk Assessment Process. Detailed methodology is in Appendix V, Section 16.2 and 16.3.

7.1.1 Malicious Actors and Acts

In today's regulatory and security environment, the main plausible threat facing high containment, research laboratories that store or study GoF viruses, involves malicious insiders who have authorized access to the laboratories and virus(s) contained therein. Insiders may work alone or in coordination with an outside group. Their motivations range from emotional disturbances to ideological radicalization by domestic and transnational terrorist organizations. The likelihood that outsiders could gain access to a laboratory without insider assistance is low. Therefore, outsiders present a threat to the periphery of the research complex or building only, but not a significant threat to the high containment laboratory itself.

7.1.2 Security Governance

Governance of infectious disease research is extremely complex, involving international agreements, domestic law, guidance, and contractual requirements in addition to institutional, local, and state-specific policies. Highly pathogenic avian influenza, the reconstructed 1918 influenza, and SARS-CoV viruses are

all Select Agents and are therefore covered by the Select Agent Regulations. Low pathogenic avian influenza and MERS-CoV are not Select Agents. Security systems, protocols, and practices at non-select agent, select agent, and Tier 1 select agent levels were reviewed to evaluate the likelihood of a malicious actor carrying out a successful act involving a laboratory that stores or studies a GoF virus. Analysis of plausible threats accounts for current security measures at the lowest level at which GoF research is conducted.

7.1.3 Qualitative Assessment: Plausible Threats

Based on historical incidents, the most likely malicious acts to be carried out in or on a laboratory that studies or stores GoF virus(s) include removal of the virus from frozen stocks, experimental samples, equipment, or research animals; deliberate contamination of personal protective equipment or laboratory equipment; deliberate compromise of the personal protective equipment or laboratory equipment; and mixing of infected with uninfected samples or animals outside proper containment. In addition, incidents involving bombs or active shooters may cause loss of containment if carried out inside or near the entrance of high containment laboratories in which GoF research is conducted. Noncompliance with security regulations and networked control systems might increase laboratory biosecurity risks. Table 7.1 summarizes these plausible threats, including both malicious actor and act.

Table 7.1. Plausible Threats Involving High Containment Research Laboratories That Store or Study GoF Viruses

Overt	Insider	Active shooter or physical assault Bomb detonated near or inside high containment space
	Outsider	Bomb detonated at building periphery
Covert Act (Expose Public)	Insider	Removal of GoF virus (frozen stock or experimental sample), infected animals, or contaminated equipment
Covert Act (Expose Laboratory Workers)	Insider	Removal of GoF virus in experimental samples Deliberate contamination of personal protective equipment or laboratory equipment Deliberate compromise of laboratory equipment or personal protective equipment Mixing of experimental samples or animals into lower containment

7.1.4 Conclusions

The existing regulatory infrastructure governing influenza, SARS-CoV, and MERS-CoV appears to provide sufficient defenses, if properly implemented, against unauthorized outsiders from accessing modified viruses. However, clarity and guidance associated with current policies could be improved to enhance compliance with required security measures. In addition, data that could be used to inform the need for additional security measures does not exist (or is not in the public domain).

Only a handful of GoF traits significantly increase biosecurity risk after a malicious event targets a laboratory. For seasonal and pandemic influenza, the ability to overcome protective vaccination and antiviral resistance modestly increases risk by increasing the potential consequences in the high income countries. There is no significant effect on risk if the global population is considered as a whole. Increasing the transmissibility and ability to evade residual immunity significantly exacerbates risk because outbreaks are more likely to occur, to escape local control and will create more consequential global outbreaks. For avian influenza, increasing transmissibility greatly increases risk because this

modification is required to spark a global outbreak of a disease by human-to-human contact, potentially infecting millions. Without this change, the hazard is restricted to those exposed to contaminated materials and infected birds, limiting the outbreak to thousands of cases at most. Increasing pathogenicity can modestly increase risk. Similarly, the wild type coronaviruses have a very small chance of sparking a global outbreak so increasing transmissibility greatly increases risk. Increasing pathogenicity can modestly increase risk.

When comparing the biosafety and biosecurity risks, a successful event that covertly infects the public (theft from an influenza laboratory of an infected animal, contaminated piece of equipment or viral stock) must occur once every 65-190 years for biosecurity event to have the same total risk as biosafety events. Given the frequency with which these malicious acts have occurred in the past, this analysis suggests that biosecurity considerations be given as much weight as biosafety issues.

7.2 Findings: Assessment of the Offense (Possible Threats to US Research Laboratories)

Incidents of criminal, terrorist, and illicit governmental activities involving pathogens, US laboratories, and/or researchers have been documented in several books, articles, official government documents, and other open source publications. However, the potential risks of intentional or accidental release of laboratory-generated or adapted pathogens into the community or environment from deliberate acts whose main goal is not bioterrorism often are not included in these accounts. Similarly, the risk that cyber breaches may result in the intentional disruption of facility operations has not been fully described in open literature. The lack of publicly available data about the likelihood that a cybersecurity breach could disrupt facility operations and control systems of high containment laboratories makes assessing such threats prohibitively difficult in unclassified settings. Therefore, the potential threats to human health that cyber breaches pose are not addressed in this report.

In our assessment of the potential biosecurity threat associated with GoF influenza, SARS-CoV, and MERS-CoV research, a variety of malicious actors, malicious acts, and consequences, including deliberate incidents that resulted in accidental release and cyber security breaches were evaluated. Furthermore, the motivations and capabilities of each malicious actor type based on conventional knowledge and historical events found in open source documents were evaluated. Finally, historical incidents of deliberate harm or application of science for destructive purposes were considered in this analysis.

The following section summarizes actual malicious acts against laboratories and health care facilities, or involving attempts to acquire pathogens based on analysis of the open-source, historical literature. Figures 7.2 – 7.4 summarize all of the historical events that occurred in the United States over the past 25 years based on open source reporting.

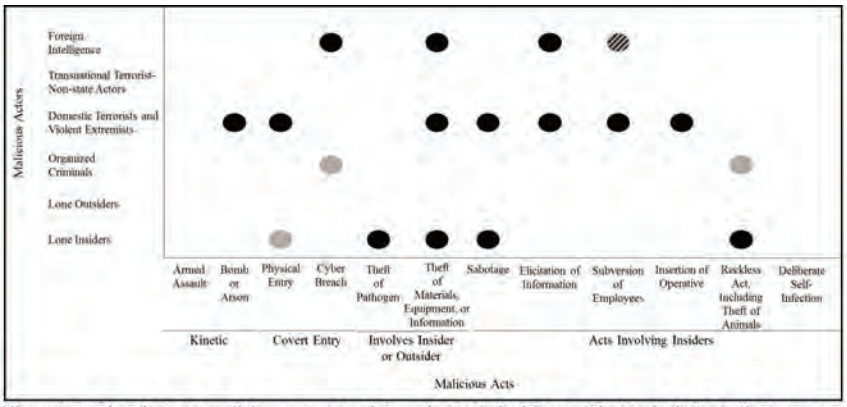

Figure 7.2. Historical acts malicious actors carried out in the United States. The black circles indicate two or more historical events, the grey circles indicate one historical event, and the cross-hatched black-grey circles indicate one or two historical events.

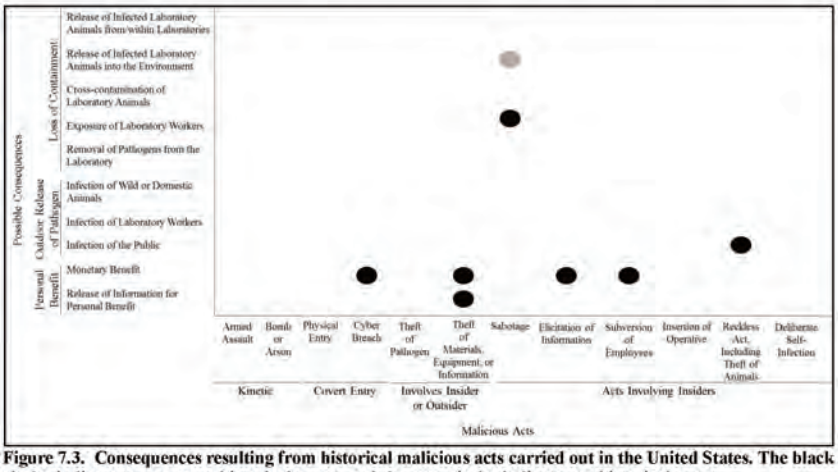

Figure 7.3. Consequences resulting from historical malicious acts carried out in the United States. The black circles indicate two or more historical events and the grey circles indicate one historical event.

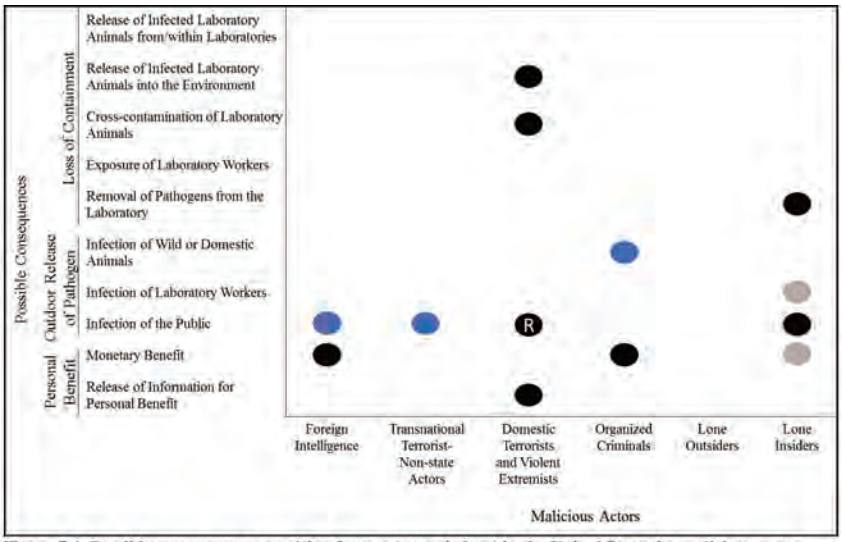

Figure 7.4. Possible consequences resulting from acts carried out in the United States by malicious actors. The black circles indicate two or more historical events, the grey circles indicate one historical event, the grey hatched circles indicate group efforts that may/may not have been associated with a criminal organization, and the blue circles indicate hopeful outcomes of planned or attempted (but failed) events. The "R" in one of the black bubbles indicates the Rajneeshee Cult, who are the only group of domestic terrorists that deliberately exposed members of the public.

7.2.1 Malicious Actors

The following key findings are reached regarding malicious actors, based on the research presented in the section above:

- Analysis of past acts by malicious actors that involved a US laboratory shows that, out of a universe of possible acts, relatively few malicious acts with the potential to lead to a breach in containment have been carried out.

- The majority of the documented prior acts have been committed by domestic terrorist and extremist groups, specifically by animal rights extremists. These groups have engaged in a wide range of malicious acts, including laboratory arson, sabotage, subversion of employees, and reckless acts such as the release of laboratory animals. Although these actions probably did not seek the release of a pathogen from a laboratory, they nevertheless have resulted in such an outcome on at least one occasion. In one case dating from 1989, animal rights extremists released 30 infected mice infected with cryptosporidium from a laboratory, probably without knowing that the mice were infected.[367] However, animal rights extremists have been rigorously pursued and

[367] "Diseased mice freed in arson fires, break-in," *Spartanburg Herald-Journal*, April 4, 1989, A2. Retrieved at: https://news.google.com/newspapers?nid=1876&dat=19890404&id=2kwsAAAAIBAJ&sjid=Vs4EAAAAIBAJ&pg=6664,1859692&hl=en.

arrested by FBI in recent years.[368,369,370,371] The number of animal rights attacks appears to have decreased in recent years because of increased security at research institutions[372] and increased arrests by law enforcement.

- Many documented events occurred before new counter-terrorism and counter-extremist laws and policies were put into place. With these new requirements in place, carrying out a malicious act today against a high containment laboratory in the United States is challenging, which might deter or prevent groups from repeating past attacks. This idea was highlighted in propaganda from one animal rights extremist group, which blamed their decreased activity against laboratories on the difficulty of penetrating "increased security."[373]

- Insiders pose a significant risk because of lone actor incidents, the unpredictability of emotionally disturbed insiders, potential for radicalization by extremists or terrorists, or elicitation or subversion incidents. Insiders have carried out or been involved in malicious acts involving the diversion of a pathogen from a laboratory and the infection of someone in the general public. In addition, noncompliance with security regulations increases the potential biosecurity risk posed by insiders.

- Transnational terrorist groups, including state-like groups, were found to be unlikely to target US laboratories directly through armed assaults or bombings. However, foreign terrorist organizations, such as al Qaeda and ISIL, have issued calls for scientists, doctors, and engineers to join their cause, which includes the use of specialized skills to inflict harm.

- Foreign intelligence entities have and continue to target biological laboratories to steal information or laboratory materials. These efforts can be done through elicitation or subversion of laboratory employees, insertion of an operative, or more recently, remotely through hacking into institutional computer networks. No information in open source literature links these incidents of theft to release of a biological agent.

- In the past, a select few foreign intelligence agencies weaponized biological agents for use in assassinations. In addition, the Soviet Union's KGB targeted Western research on modified pathogen strains, possibly to bolster the Soviet offensive program.[374]

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368 John E. Lewis, Deputy Assistant Director, Federal Bureau of Investigation, Testimony before the Senate Judiciary Committee, Washington DC., U.S.A., May 18, 2004, https://www.fbi.gov/news/testimony/animal-rights-extremism-and-ecoterrorism.

369 Moran R, "Animal activists defend tactics that led to raid – Protests target the homes of business executives," *The Inquirer*, November 22, 2004, http://articles.philly.com/2004-11-22/news/25379045_1_huntingdon-life-sciences-animal-activists-animal-rights.

370 Law enforcement efforts outside of the U.S. have also targeted animal rights extremists in recent years. Mark Oliver, "30 arrested as raids target animal rights extremists," *The Guardian*, May 1, 2007, http://www.theguardian.com/uk/2007/may/01/animalwelfare.world.

371 Patrick Sawer, "Debbie Vincent: Former soldier turned animal rights extremist jailed for six years," *The Telegraph*, April 17, 2014, http://www.telegraph.co.uk/news/uknews/crime/10772486/Debbie-Vincent-Former-soldier-turned-animal-rights-extremist-jailed-for-six-years.html.

372 The following extracts from a website maintained in support of the Animal Liberation Front, a domestic extremist animal rights organization, supports this claim: "Numerous larger liberations took place in the early eighties before technologically advanced security systems were placed in most larger animal laboratories" and, "Because of increased security, liberations haven't been as frequent in the 1990's [...]" "Laboratory Animal Liberation Campaign," Animal Liberation Front, http://www.animalliberationfront.com/ALFront/lab.htm.

373 Ibid.

374 Leitenberg M., Zilinskas R., (2012) *The Soviet Biological Weapons Program: A History*. Cambridge, MA, Harvard University Press

- Organized criminals have not attempted to steal pathogens from a US laboratory. However, one case of cyber-crime suggests that theft of information on applied life science research can be lucrative, and therefore tempting, for organized criminal groups.

- Lone outsiders do not pose a significant threat to research laboratories, especially Biological Select Agent and Toxin laboratories, because they do not have access to the facilities. By definition, these actors are not working with an insider and would not have opportunities to gain access to facilities in the absence of intentional or unintentional assistance[375] of an insider.

Interviews confirmed the following threats of concern:

- **Insiders** with access to information and pathogens and who become discontented or disgruntled, radicalized, or elicited or subverted are a security concern.

- **Transnational terrorists** who are interested in biological weapons are a security concern.

- **Domestic extremists**, such as animal rights extremists, anti-vaccine extremists, and eco-radical groups, who see harming researchers and institutional administrators, and/or vandalizing institutional facilities as a useful approach to convey their messages are a security concern The threat posed by domestic extremists appears to vary by the laboratory's location.

- **Lone outsiders** do not raise much concern because they are not working with an insider and have difficulty accessing laboratories and breaching facility defenses unassisted.

- **Active shooters** on university campuses are of significant concern even though **no** incidents involving an active shooter in a high containment laboratory have been described.

7.2.2 Malicious Acts

The following key findings are reached regarding malicious acts, based on the research presented in the section above:

- Armed assaults at laboratories have not occurred previously, but with increasing incidents of active shooter cases on university campuses, the potential for armed assault might exist.

- A bombing attack against a US lab has not taken place. Because malicous actors have bombed hospials and related facilities, this type of attack remains possible.

- No exposure or release events have occurred from sabotage, despite the relative frequency of sabotage incidents.

- Reckless acts provide the greatest opportunity for an outbreak to occur, and have occurred on several occasions in the past, as documented above.

- A deliberate infection of a member of the public through a deliberate or reckless act by an insider represented the most common pathway for loss of containment across the spectrum of malicious actors and acts considered.

[375] An example of unintentional assistance by an insider is access through an insider not complying with physical, information, or transportation security measures.

- A reckless act involving the release of an infected animal outside of containment has occurred once before, as a result of an attack by a domestic animal rights extremist group who likely did not know the mice were infected.

- Deliberate self-infection remains a hypothetical concern. The closest event documented in open source literature is one reported HIV self-infection case involving an outsider without lab access who attempted suicide, probably with the help of an infected friend.[376]

- Interviews confirmed cyber breach of computer networks and cyber security issues are a significant concern particularly because they have resulted in several incidents of information theft. Furthermore in the early 2000s, a computer worm infected the software of an Iranian uranium enrichment plant, in addition to other industrial sites, affecting operations of Iranian nuclear centrifuges.[377,378] The Department of Defense (DOD)'s Defense Science Board Task Force considered the potential threat of cyber-sabotage in their May 2009 assessment of DoD laboratory security, and recommended that an in-depth study be conducted to determine the potential cyber threat against US laboratories.[379]

A summary of the findings drawn from open source literature is presented in Figure 7.5 which highlights combinations of malicious actors, acts, and consequences of malicious acts based on historical incidents (in green, red, and red/green circles) and identifies possible combinations based on an evaluation of malicious actor motivation and capability (blue, blue hatched, or blue diagonal circles). This summary of findings will be described in detail in the following sections.

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376 This case is described in: Seth Carus W. (1998) *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900* Washington, DC, National Defense University.

377 Kushner D, The Real Story of Stuxnet. IEEE Spectrum. Accessible at: http://spectrum.ieee.org/telecom/security/the-real-story-of-stuxnet. Accessed on November 4, 2015.

378 Langner R. To Kill a Centrifuge: A Technical Analysis of What Stuxnet's Creators Tried to Achieve. Accessible at http://www.langner.com/en/wp-content/uploads/2013/11/To-kill-a-centrifuge.pdf. Accessed on November 5, 2015.

379 Office of the Under Secretary of Defense For Acquisition, Technology, and Logistics, Defense Science Board, "Report of the Defense Science Board Task Force on Department of Defense Biological Safety and Security Program," May 2009, p. xii, 18-19, 41, <http://www.acq.osd.mil/dsb/reports/ADA499977.pdf>

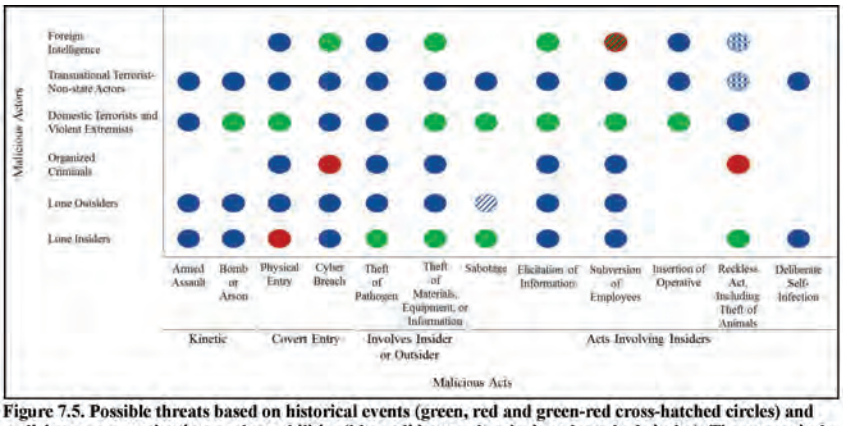

Figure 7.5. Possible threats based on historical events (green, red and green-red cross-hatched circles) and malicious actor motivations and capabilities (blue solid, cross-hatched, and patched circles). The green circles indicate two or more historical events, the red circles indicate one historical event, and the cross-hatched green-red circles indicate one or two historical events. The blue circles indicate possible threats based on malicious actor motivation and capability. The patched blue circles indicate planned or failed attempts. The cross-hatched blue circles indicate limited possibility.

7.2.3 Detailed Descriptions

Detailed descriptions of malicious acts perpetrated by various malicious actors (lone outsider, lone insider, organized criminals, domestic terrorists and violent extremists, transnational terrorist non-state groups, and foreign intelligence entities) are included in Appendix V to this report:

Section 16.4: Analysis of Malicious Actor Motivations and Capabilities
Section 16.5: Detailed Analysis of Historical Incidents
Section 16.6: Attacks Against Laboratories
Section 16.7: Biocrimes Committed by Individuals
Section 16.8: Terrorist and Extremist Events Tied to Biological Warfare (BW)
Section 16.9: Designated Foreign Terrorist Organizations and BW
Section 16.10: Detailed History of Known Terrorist BW Programs
Section 16.11: Other Terrorist Groups Linked in Some Fashion to BW
Section 16.12: Islamic State in Iraq and the Levant (ISIL) Group Overview

Some types of successful incidents may go undetected, and in general, incidents may be tied to sensitive law enforcement and intelligence information. Hence, open source reporting alone is unlikely to lead to a complete list of all relevant historical incidents. Historical patterns can be disrupted, for instance, as a result of the widespread implementation of new security programs, by the arrest of key group members, and by the emergence of new malicious actors. Malicious actors may decide, in line with shifts in motives and capabilities, to change the way they operate and to select new targets. To address the potential shortcomings of relying solely on historical data, hypothetical events are also considered in light of the motivations and capabilities of each malicious actor type. That is, when no historical case has been identified for a particular actor-act pairing, an argument is presented to explain why that pairing is unrealistic or, on the contrary, for why it cannot be discounted. These cases are called hypothetical.

7.3 Findings: Defense Assessment

An assessment of the overall risk posed by malicious actors necessitates an evaluation of the current governance structure for biosecurity and related policies and the implementation of security measures at research institutions. The historical and operational links between safety and security highlight the need to include both in this evaluation.[380] In addition, the agents associated with the Deliberative Process – influenza, SARS-CoV, and MERS-CoV – are subject to different requirements. Highly pathogenic avian influenza and SARS-CoV are select agents subject to the Biological Select Agents and Toxins Regulations.[381] Although no GoF viruses are currently Tier 1 BSAT, a recent Notice of Proposed Rule-Making has asked for public input on the upgrade of laboratory-generated, mammalian-transmissible H5 influenza viruses (specifically, those viruses that are contain the HA from the A/Gs/Gd/1/96 lineage and made transmissible among mammals by respiratory droplets in the laboratory) to the Tier 1 level of Biological Select Agents and Toxins.[382] Low pathogenic influenza and MERS-CoV are not classified as Biological Select Agents and Toxins. For this reason, included in this analysis are security measures, whether from governing documents and practices on safety or security, at the non-select agent, select agent, and Tier 1 select agent levels.

7.3.1 Overview of Security Measures

Table 7.2 below summarizes specific requirements applicable for all laboratories depending on their biosafety level (second column), additional requirements enforced at laboratories working with Select Agents and Toxins (third column), and additional requirements enforced at laboratories working with Tier 1 Select Agents (fourth column). The second column constitutes the base level of security, and each column thereafter lists additional security requirements. The September 2014 institutional DURC oversight policy applies to select agent and Tier 1 select agent laboratories and to non-select agent laboratories conducting research with *di minimus* quantities of botulinum toxin.

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380 Biosafety measures mitigate risk of *accidental* exposure to hazardous biological agents, such as lab acquired infections and environmental exposure. Biosecurity measures mitigate risk of *intentional* theft or misuse of biological samples or relevant sensitive information. U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, December 2009, p. 105, http://www.cdc.gov/biosafety/publications/bmbl5/.

381 42 C.F.R §73, 9 C.F.R. §121, and 7 C.F.R. §331.

382 Proposed regulation covers laboratory generated, mammalian, respiratory-transmissible influenza viruses containing the hemagglutinin from the A/Goose/Guangdong/1/96 lineage. Federal Register Volume 80, Number 136, Pages 42079-42084 http://www.gpo.gov/fdsys/pkg/FR-2015-07-16/html/2015-17435.htm.

Table 7.2. Security-Specific Requirements in General, Select Agent, and Tier 1 Select Agent Labs

Topic	General	Select Agent (in addition to General)	Tier 1 Select Agent (in addition to Select Agent)
Personnel Training	• Appropriate biosafety training • Dual use research of concern training (for research involving *di minimus* quantities of botulinum toxin that is considered to be DURC)	• Security training, at least annually • Dual use research of concern training (for research assessed as DURC)	• Insider threat awareness training • Stricter personnel reliability reporting
Personnel Reliability	• DoC and/or DoS permits for pathogen access by foreign nationals (if needed)[1] (Reference checks for new hires; optional)	• Individual security risk assessment • Suspicious activity reporting process • Separate criminal background check (optional) • DoC and/or DoS permits for pathogen access by foreign nationals (if needed)[1]	• Pre-access suitability assessment • Formal continuous suitability assessment (Behavioral threat assessment teams optional)
Physical Security	• Self-closing lockable doors (BSL-2 and up, all animal) Separate space from traffic flow, doors locked (BSL-3 and up, all animal) • Separate building or zone, locked doors (BSL-4, ABSL-4) • Self-closing doors (animal) • Sealed (BSL-3 and up) and break-resistant (BSL-4) windows • (Windows not recommended for ABSL vivarium; optional) (ID badges, access control, "normal" working hours; optional) (Electronic cardkey access; optional)	• Physical security in security plan • Procedures to remove potential malicious actors • Reporting potential crimes or access control issues • Access control management • Inspection of suspicious packages • Escort visitors	• Three security barriers, one monitored • Access control on final barrier • Backup power for access control systems • Response time at or under 15 minutes, or physical barriers adequate to hold until responders arrive Restricted off-hours access even for approved staff • Procedures for visitors, their property, and their vehicles

Table 7.2. Security-Specific Requirements in General, Select Agent, and Tier 1 Select Agent Labs

Topic	General	Select Agent (in addition to General)	Tier 1 Select Agent (in addition to Select Agent)
Surveillance and Monitoring	• Access controls and training requirements • Alarmed exits (BSL-4, ABSL-4) • Occupational health monitoring (BSL-4, ABSL-4, lower levels by risk assessment • Ventilation alarms (BSL-3 and up, optional below level 4) • Facility video surveillance (optional, generally not monitored) • Yearly facilities inspection – biosafety cabinets, HVAC	• No additional requirements	• Intrusion detection systems • Occupational health monitoring
Storage, Inventory, and Accountability Processes	• General inventory and material management process for biological stocks (optional) • Record entry/exit in logbooks (BSL-4, ABSL-4)	• Record number of containers, storage location, and chain-of-custody information for long-term storage • Record animal counts, species, location, and final disposition • Access records in logbooks • Access control to inventories • Inventory audits after moving, PI turnover, or theft/loss • DoC and/or DoS permits for pathogen export[1]	• More stringent reviews, logs, and inventory audits (optional)
Transfer, Shipment, and Chain-of-Custody Protocols	• Triple package agents[2] o Labeling requirements for air shipment[2] o Import permit • (CDC, USDA), interstate permit (USDA), potential need for interstate transfer permits for imported samples (CDC, case-by-case) [2] • DoC and/or DoS permits for pathogen export (if needed) [1]	• Shipping permits from CDC/APHIS required[2] o Report receipt or loss/theft/delay to CDC/APHIS within 48 hours[2] o Report damage to CDC/APHIS immediately[2] • Record transfers • DoC and/or DoS permits for pathogen export[1]	• No additional requirements

Table 7.2. Security-Specific Requirements in General, Select Agent, and Tier 1 Select Agent Labs

Topic	General	Select Agent (in addition to General)	Tier 1 Select Agent (in addition to Select Agent)
Emergency Response Protocols	• External communication capability (BSL-4, ABSL-4) • Emergency access and egress plans (BSL-4, ABSL-4) • Plans for man-made or natural disasters (Animal)	• Annual drills to test emergency and incident response plans	• Security response time at or below 15 minutes, or physical barriers adequate to hold until responders arrive

General from BMBL[3] unless noted. Select Agent and Tier 1 Select Agent from Select Agent regulations[4] unless noted.
[1]US Department of Commerce, "Deemed Exports and Fundamental Research for Biological Items";
15 CFR 734.3-8, "Scope of the Export Administration Regulations";
15 CFR 744.4-6, "Control Policy: End-User and End-Use Base";
US Department of Commerce, Commerce Control List, "Category 1 – Special Materials and Related Equipment, Chemicals, 'Microorganisms' and 'Toxins'";
22 CFR 121.1(XIV)(b), "The United States Munitions List."
[2]49 CFR 175.134, "Class 6, Division 6.2 – Definitions and exceptions";
49 CFR 173.196, "Category A infectious substances";
49 CFR 173.199, "Category B infectious substances";
49 CFR 172, "Subpart I- Safety and Security Plans";
9 CFR 122, "Organisms and Vectors."
42 CFR 71, "Foreign Quarantine"
[3]US Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition.
[4]42 CFR 73, US Government Publishing Office, "Select Agents and Toxins"
9 CFR 121, US Government Publishing Office, "Possession, Use, and Transfer of Select Agents and Toxins"

7.3.2 Detailed Descriptions

A detailed analysis of the requirements, implementation practices, and current gaps in security measures is provided in Appendix V of this report.

Section 16.13: Biosafety and Biosecurity at US Research Laboratories
Section 16.14: Laws, Guidance, Policies, Practices, and International Agreements on Biosafety and Biosecurity
Section 16.15: Restriction of Fundamental Research, Dual Use Research of Concern, and NIH Guidelines for Recombinant DNA
Section 16.16: Analysis of Security Measures
Section 16.17: Major Challenges and Knowledge Gaps

7.4 Analysis of Offense and Defensive Measures

The biosecurity risk assessment presents a semi-quantitative evaluation about whether deliberate acts involving GoF influenza, SARS-CoV, or MERS-CoV will result in a local outbreak or pandemic. The assessment involves: 1) qualitative analysis of plausible threats facing institutions that conduct GoF influenza virus, SARS-CoV, or MERS-CoV research based on systematic evaluation of historical

incidents, malicious actor motivations and capabilities, and implemented security measures at US research institutions and 2) quantitative analysis of the potential for the plausible threats to cause infections in the local community or broader and of the comparison of possible pandemic consequences of plausible threats involving GoF influenza virus, SARS-CoV, or MERS-CoV and non-GoF viruses. Although an actual or attempted biosecurity incident could cause significant damage to research progress, national preparedness and response efforts, the nation˜s economy, or socio-political situation, the assessment focuses on the consequences to human health (both illness and death) at the individual (i.e., laboratory worker, malicious actor, or emergency personnel) and population (i.e., local or global communities) levels should a pathogen be removed from containment deliberately or accidently.

No unclassified information describing the threats to research laboratories that store or study GoF influenza, SARS, or MERS-CoV virus is available. Therefore, to identify the types of acts that may target a GoF laboratory, our approach involved examining historical incidents involving life science laboratories and hospitals, evaluating the motivations and capabilities of malicious actors, and determining if and how existing security measures affect the likelihood of success of a given malicious act. All of the data collected on potential threats and biological security governance were used to assess the plausible threats facing laboratories that study or store GoF virus(s).[382] For the purpose of this analysis, "plausible threats" are defined as the most probable events that could lead to a loss of containment from a biosecurity incident. Therefore, the analysis focused on the plausible threats assessed within the current context of laboratory security and their potential to lead to localized or widespread infections.

The malicious acts that present the greatest risk to human health are assessed sequentially, starting with malicious actors. The most plausible actors are further evaluated by the most probable and consistent malicious acts they may commit. Finally, the most likely immediate consequence of a probable and consistent act that has been committed is evaluated. At each step, probable threats are evaluated within the context of current security measures at US high containment research laboratories. The final result is the most plausible threats based on evaluation of historical data, consistency with malicious actor motivation and capability, and likelihood within the current security environment at high containment research laboratories in the United States.

The potential of plausible malicious acts to cause global pandemic was assessed using the biosafety risk assessment models. By leveraging the biosafety risk assessments to analyze biosecurity risk, the important input parameters become the number of initial infections and response time after an incident (including emergency response and/or public health response). Deliberate and accidental risks result in vary similar outcomes and this approach allows for comparisons to be made between biosafety and biosecurity risks that cause similar human health outcomes.

7.4.1 Qualitative Assessment of Plausible Threats

7.4.1.1 Malicious Actor

Analysis of malicious actor intent falls into two categories: 1) intent to target US research institutions to acquire GoF viruses for use as weapons; and 2) intent to harm US research institutions and/or laboratory workers, but not through the weaponization of pathogens stored or studied in research laboratories. Table 7.3 summarizes the likelihood that the malicious actors considered have the intent, capability and ability to access laboratories with respect to each of these categories. The analysis is based on an evaluation of historical cases and malicious actor motivations and capabilities described in Section 7.4 and Appendix V

[382] Noncompliance with security regulations might increase biosecurity risk intentionally or unintentionally. However, because no repository of tested best practices exist, some requirements may not be easily implemented at research institutions given building design, institutional policies, and local and state laws.

Section 16.2-16.9 and the safety and security measures included in Section 7.5 and Appendix V Section 16.10-16.11. Although this assessment is grounded in historical incidents, incorporation of malicious actor motivations and capabilities ensured that plausible incidents that have not previously occurred would be considered. Of greatest relevance to the discussion about actors is their definition: outsiders are not authorized to access high containment research laboratories in which GoF research with influenza, SARS-CoV, or MERS-CoV are conducted, while insiders are authorized to access such laboratories by definition. The approach taken in this analysis can be applied to biosecurity risk assessments of research involving other pathogens.

Table 7.3. Malicious Actor Intent, Capability, and Opportunity

	Deliberate Acts that Use of Pathogens as Weapons			Deliberate Act Resulting in Accidental Release of Viruses		
	Intent to Acquire Virus to Use	**Capability to Acquire Virus**	**Ability to Access Laboratory**	**Intent to Carry Out Malicious Act**	**Capability to Carry Out Malicious Act**	**Ability to Access Laboratory**
Foreign Intelligence Agencies						
Transnational Terrorists, non-state actors						
Domestic Terrorists and Extremists						
Organized Criminals						
Lone Outsiders						Only to outside of building
Lone Insiders						

Black *indicates consistency of intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Dark ***Grey*** *indicates possible intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Grey *indicates inconsistency of intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

7.4.1.1.1 Deliberate Act at US Research Laboratory to Use Pathogen as Weapon

The most likely actors with **intent** to target US research institutions to acquire GoF influenza virus, SARS-CoV, or MERS-CoV for use as weapons are transnational terrorists and lone insiders.

- Although foreign intelligence agencies may want to acquire viruses, their purpose for doing so is likely for intelligence, scientific advancement in their home countries, or commercial benefit.

Although the possibility that foreign intelligence agencies may want to acquire GoF virus to incorporate into their offensive biological weapons cannot be ruled out, the likelihood of this intent is low. Approximately 172 countries are party to the Biological Weapons Convention, which bans development and stockpiling of biological weapons, and all nations are required to abide by United National Security Council Resolution 1540, which requires countries to implement and enforce measures preventing proliferation of biological weapons within their borders. These international obligations decrease (but does not eliminate) the likelihood that Nations acquiring a virus for an offensive biological weapons program.

- Translational terrorists, specifically al Qaeda, continue to express interest in acquiring pathogens for use as weapons. In addition, al Qaeda and ISIL have recruiting efforts that target individuals with technical skillsets to join their causes and undertake malicious acts within their means. However, no available information suggests that these groups have recruited scientists in the US or that other transnational terrorist groups have interest in biological weapons. For these reasons, the intent of transnational terrorists is described as possible.

- In our analysis, individuals who self-radicalize and plan to or carry out a malicious act in the absence of a formal affiliation with a terrorist organization are considered Lone Outsiders or Lone Insiders. This distinction is made because the level of resources, support, and success afforded a member of a group compared to an individual acting alone is different, all of which will be described in the analysis of capabilities, access, and likelihood of malicious acts.

- Historically, members of domestic terrorist groups, but not animal rights extremist or eco-radical groups, have sought to acquire bacteria from culture collections. Similarly, recent policy debates about synthetic genomics have raised concerns that individuals, some of whom may be members of these groups, may seek to acquire viral DNA from DNA synthesis companies to recreate viruses. However, no available examples exist describing cases where domestic terrorist groups have sought to steal or have successfully stolen viruses from high containment laboratories in the United States, suggesting the intent to do so is inconsistent with their motivations. However, one example exists describing a domestic terrorist group that used an agent against the public to sway a local election (Rajneeshee Cult).

- Organized criminals and lone outsiders are not likely to be interested in stealing virus from a high containment laboratory based on the lack of open source examples of such incidents and our understanding of the motivations of organized criminals.[384]

- Several historical cases involving lone insider theft and use of bacteria to harm others were identified in open source literature during our study. These cases have involved disgruntled, dissatisfied, disturbed, or radicalized insiders who remove a pathogen from a laboratory to infect co-workers, spouses, or family members. Extrapolating these cases to GoF viruses, the possibility that a lone insider, with malicious intent, may acquire virus from a research laboratory to use as a weapon is high.

When divorcing intent from capability, the most likely malicious actors to have the **capability** to acquire GoF influenza virus, SARS-CoV, or MERS-CoV from US research laboratories are foreign intelligence entities, an insider acting alone or in concert with any category of malicious actors, and lone insiders.

[384] Historical examples of lone outsiders acquiring bacteria from culture collections do exist. But, to our knowledge, culture collections do not have GoF influenza, SARS-CoV, or MERS-CoV viruses.

- Many foreign intelligence agencies have the resources and levels of expertise to suggest they have a high level of capability. In addition, these agencies are known to elicit information from insiders as part of their typical tradecraft.

- Transnational terrorists, domestic terrorists, and domestic extremists themselves are unlikely to have the requisite capability to illicitly acquire a virus from a high containment laboratory in the United States. However, documented historical cases describing insider recruitment suggest that insider-assisted capability is likely.

- Organized criminals and lone outsiders are unlikely to have the capability to steal virus from a high containment laboratory, particularly since no open source examples exist. However despite the lack of motivation, the possibility that organized criminal groups force insiders to assist in acquiring virus cannot be ruled out.

- Lone insiders present the highest risk when considering capability because they have the knowledge, skills, and access necessary to acquire and/or manipulate the viruses.

When considering an actor's **ability to access** a GoF influenza virus, SARS-CoV, or MERS-CoV in a high containment research laboratory in the United States, the most likely malicious actors to have access are foreign intelligence entities, an insider acting alone or in concert with any category of malicious actors, and lone insiders.

- Research institutions supporting animal research and high containment research have access controls in place to protect against unauthorized access to the laboratories. These controls can take the form of guards; electronic, biometric, or mechanical intrusion prevention/detection systems; and/or some combination of these measures. Based on these access controls, along with periodic monitoring of access to laboratories, the likelihood that any outsider, who is not working with an insider, could gain access to the high containment, research laboratory to acquire GoF virus is extremely low. That said, these security measures are only as good as the community who observe them (i.e., noncompliance with security regulations might increase insider-assisted biosecurity risks).

- Foreign intelligence agencies are known to elicit information and materials from insiders as part of their typical tradecraft, suggesting the possibility of gaining access to GoF viruses through indirect means. In addition, these agencies may have personnel who are authorized entrance into the laboratories and have direct access to GoF viruses (e.g., operative or elicited individual). Professionals likely would not be identified by currently implemented personnel reliability measures at research institutions or deterred by access control measures.

- Acting alone, translational terrorists are not likely to have any opportunities to acquire virus in high containment research laboratories in the United States. However, these organizations are known to recruit individuals to their causes, suggesting that they could acquire virus with the help of an insider (e.g., through elicitation, subversion, or recruitment). Current insider threat training and personnel reliability measures that allow for periodic behavioral assessment, specifically implemented for Tier 1 BSAT, and non-punitive reporting of changes in co-worker behavior (any level of research) could alert institutional officials to possible insider radicalization. However, colleagues may not recognize such changes or may be in denial that such changes are taking place in a friend or colleague, limiting the effectiveness of personnel security measures.

- Domestic terrorists, domestic extremists, organized criminals, and lone outsiders are not likely to have access to virus stored in high containment, research laboratories in the United States. Despite the lack of access, the possibility that domestic terrorists, domestic extremists, and organized criminals could acquire a virus with the help of an insider cannot be ruled out. Historical examples of domestic extremist groups gaining access to lower containment laboratories and eliciting information about facilities or attempting to get into animal facilities exist. However, the likelihood that such elicitation and access attempts would translate to acquisition of GoF virus is low in light of current access controls for high containment research laboratories, particularly BSAT laboratories, and personnel reliability measures for Tier 1 BSAT laboratories. Increased insider vigilance and non-punitive reporting would further decrease the likelihood of insider-assisted acquisition.

- Lone insiders are extremely likely to have opportunities to acquire virus from high containment research laboratories because they have authorized access to these laboratories. The monthly inventory checks on stored pathogens would not necessarily deter insiders (both lone insiders and insiders assisting groups) from removing virus from the laboratory. In addition, the identification of missing virus may be impossible if some virus is removed from a vial that remains in the freezer. In addition, inventory checks would not identify removal of virus from experimental samples.

- The possibility that an actor could steal pathogen during transportation appears to be low because GoF viruses apparently are not shipped.[385] Even if a virus was shipped, specific information about shipping dates, trucks, and vendors are not accessible to outsiders.

When evaluating intent, capability, and opportunity together, the most likely malicious actor to target a US research laboratory to acquire a pathogen for use as a weapon is an insider, either working alone or in coordination with a group, likely a transnational terrorist group.[386]

7.4.1.1.2 Deliberate Act at US Research Laboratory Resulting in Accidental Release of Viruses

Deliberate acts directed towards institutions and people, but not conducted for explicit acquisition of virus for use as a weapon, could target: 1) the research laboratory in which GoF research with influenza virus, SARS-CoV, or MERS-CoV is being conducted; 2) space outside the laboratory but inside the building which houses the laboratory; or 3) the area outside the facility in which the laboratory is housed. Such targeting could cause accidental release of virus from the laboratory. Specific acts associated with such targets could include armed assault, arson, bombing, vandalism and sabotage of facilities, tampering with experiments, and theft of materials, equipment, and animals.

The most likely malicious actors with the **intent** to carry out such acts include domestic terrorists and extremist groups, and lone insiders.

- The possibility that a foreign intelligence agency would carry about a deliberate act on a US research laboratory is low, especially since an attack could be construed as an act of war. This is particularly true for overt attacks, such as bombing, armed assault, or vandalism. However, the possibility that a foreign intelligence agency could accidently release a GoF virus through theft of materials or equipment cannot be ruled out.

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[385] Scientists use reverse genetics to make GoF viruses instead of shipping them according to the scientists who were interviewed.

[386] An insider also is the most likely actor to acquire a virus for non-weapons purposes, such as personal or monetary benefit or assisting foreign intelligence agencies.

- No open source information exists indicating an intent by transnational terrorists to carry out a deliberate, malicious act on high containment research laboratories in the United States. However, the possibility that transnational terrorists may want to bomb a building cannot be ruled out because of the high prevalence of such tactics by several of these groups.

- No open source information exists indicating an intent by organized criminal groups to carry out a malicious act on or in high containment research laboratories in the United States. Organized criminals are driven by financial gain suggesting that the possibility that a criminal organization might seek to sell laboratory equipment for profit cannot be ruled out. However, the relative availability of common life science equipment for online purchase decreases the likelihood that an organized criminal organization will steal from a high containment laboratory in the United States.

- Several historical cases involve deliberate acts caused by domestic terrorists and extremists who have vandalized buildings, tampered with experiments in lower containment laboratories, released research animals into the wild or their own homes, or detonated bombs near buildings. The frequency with which these groups attack research institutions for ideological purposes indicates a high likelihood that they could carry out more such deliberate acts. However, additional security measures put in place under various regulations make such acts much more difficult to plan and carry out.

- Historical cases involving the use of bombs or armed assault in public areas and at hospitals by lone outsiders exist in open source literature. However, no open source information was identified about the targeting of US research laboratories by lone outsiders. Despite the lack of motivation, the possibility that a lone outsider would detonate a bomb or carry out an armed assault outside or in a research building cannot be ruled out. However, the motivation for such an attack is not clear.

- Historical examples of lone insiders tampering with experiments for personnel benefit or theft of virus for commercial benefit suggest the presence of clear motivations for lone insiders to carry out deliberate acts, some of which could result in accidental release of virus. Consequently, the likelihood of such acts is high.

When considering only **capability**, the most likely malicious actors to carry out deliberate acts that might result in accidental release of virus are foreign intelligence agencies, transnational terrorists, domestic terrorists and extremists, and lone insiders.

- Foreign intelligence agencies are expected to have the resources, tools, and expertise needed to carry out deliberate acts that could result in accidental release of GoF virus. In addition, these agencies are known to elicit information and materials from insiders as part of their typical work.

- No open source information about transnational terrorists targeting US research laboratories exists. However, the 2001 attacks and the violence carried out by individuals who may have been radicalized by transnational terrorists suggests their capability to carry out armed assault, arson, and bombing within the United States. Whether surveillance and monitoring of building perimeters would deter or prevent a transnational terrorist or sympathizer from carrying out an attack using these tactics is unclear.

- Domestic terrorist and extremist groups have bombed hospitals, released animals from research laboratories, vandalized research laboratories and equipment, and tampered with experiments.

Their proven ability to damage the building exteriors, damage low containment laboratories, steal animals, and tamper with experiments suggests they are capable of damaging buildings in which GoF research is being conducted. However, domestic terrorist and extremist groups are not likely to carry out deliberate acts inside a high containment research laboratory without the assistance of an insider with access. Increased security, including surveillance and monitoring of building perimeters and animal facilities, and increased arrests has decreased deliberate, violent acts involving animal rights extremists. Whether this extrapolates to other domestic terrorist or extremist group is unclear.

- No open source information exists about the capability of organized criminals to damage biological research facilities in the United States deliberately. Despite this unknown capability, organized criminals could use armed assault to gain access to the facility, but the exact purpose of doing so is unclear.

- Lone outsiders have detonated bombs in public areas and at certain clinics suggesting their potential capability to damage buildings in which GoF research is being conducted. Surveillance of building perimeters may deter lone outsiders from carrying out such acts. However, the increasing number of active shooter incidents at US facilities and educational institutions suggests that such actors are not deterred by surveillance and other similar measures.

- Lone insiders are expected to have the knowledge and skills to deliberately compromise or tamper with equipment and experiments. Furthermore, historical cases involving lone insiders who tamper with co-workers' experiments are described in open source literature. These cases and the presumed knowledge and skills of lone insiders suggest that these actors likely have the requisite capabilities to carry out deliberate acts in a high containment research laboratory. Non-punitive peer reporting of unusual incidents or repeated experimental findings, damaged equipment and facilities, and behavioral changes or unusual behavior of individuals with authorized access to high containment, research laboratories are the only measures that exist to prevent or mitigate a deliberate act carried out by an insider with trusted access.

When analyzing an actor's ability to access a high containment, research laboratory, the most likely malicious actors to carry out deliberate acts that could result in accidental release of virus are foreign intelligence agencies, transnational terrorists, domestic terrorist and extremist groups, and lone insiders.

- Research institutions supporting animal research and high containment research have access controls in place to protect laboratories. These controls can take the form of guards; electronic, biometric, or mechanical intrusion prevention systems; and/or some combination of these measures. Based on these access controls and periodic monitoring of access to laboratories, the likelihood that any outsider, who is not working with an insider, would gain access to the high containment, research laboratory to tamper with experiments involving GoF viruses or their wild type counterparts is low.

- Foreign intelligence agencies are known to elicit information from insiders as part of their typical tradecraft, suggesting the ability to achieve indirect access to laboratory materials. In addition, these agencies may have personnel who can gain authorized entry to the laboratories (i.e., insertion of an operative) for direct access to high containment laboratories. Professionals likely would not be identified by currently implemented personnel reliability measures at research institutions or deterred by access control measures. Foreign intelligence agencies also may have the resources to access laboratories remotely by hacking into laboratory computer systems.

Finally, foreign intelligence agencies may have the resources to detonate a bomb or carry out an armed assault, but as previously stated, these acts could be construed as an act of war.

- Transnational terrorists may have access to the exterior perimeter of the building in which a laboratory is located and potentially to the research laboratory itself, if access control measures are insufficient. However in general, such actors would not have access to the high containment laboratory itself unless assisted by an insider. Current personnel reliability measures that allow for periodic behavioral assessment, specifically implemented for Tier 1 BSAT, and non-punitive reporting of changes in co-worker behavior (any level of research) could alert institutional officials to possible radicalization of an insider. That said, colleagues may not recognize such changes or may be in denial that such changes are taking place in a friend or colleague.

- Historically, domestic terrorist and extremist groups, such as animal rights extremists, have recruited, elicited information from, and subverted insiders to gain access to animal facilities. In addition, other groups have elicited information about clinics as they prepared to bomb buildings based on historical examples. Domestic terrorist and extremist groups are likely to access the perimeters of buildings and low containment research laboratories, but not likely to access high containment research laboratories without the assistance of an insider.

- The likelihood that criminal organizations and lone outsiders would have access to high containment, research laboratories is low. However, the possibility that an insider could assist a criminal organization in carrying out a deliberate act in a high containment laboratory cannot be ruled out, though the exact purpose behind such an act is not clear.

- Lone insiders are extremely likely to have opportunities to tamper with experiments, release animals, compromise equipment, detonate bombs, and carry out armed assault in high containment, research laboratories because they have authorized access to these laboratories.

7.4.1.1.3 Malicious Actor Conclusion

When evaluating the intent, capability, and the ability to access laboratories in which GoF research with influenza virus, SARS-CoV, or MERS-CoV, the most likely malicious actors to target a US research laboratory to carry out a deliberate act to the building perimeter are domestic terrorists and extremists, transnational terrorists, lone outsiders, and lone insiders. Although no open source information indicates whether these malicious actors are motivated to damage buildings in which GoF viruses are stored or studied, historical examples of attacks involving other types of buildings do exist.

When looking at all three components together for deliberate acts carried out inside a high containment research laboratory in which GoF research with influenza virus, SARS-CoV, or MERS-CoV is conducted, the most likely malicious actor is an insider, working alone or in coordination with a group, particularly domestic terrorist or extremist groups.

7.4.1.2 Malicious Acts and Likelihood of Escape of GoF Virus

The likelihood of success of malicious acts and resulting virus escape are based on the degree of access to a high containment research laboratory. These laboratories (i.e., biosafety levels 3 and 4) have a variety of security measures in place to prevent unauthorized access by individuals who are not approved to work and/or do not demonstrate competency and proficiency in working safely and competently in the laboratory. In addition, researchers working with BSAT are subject to review by the Security Risk Assessment (Appendix V Section 16.11.2) and those working with Tier 1 BSAT must undergo periodic

screening assessments. Based on these physical and personnel security measures, the analysis of malicious acts is divided into: 1) acts that can be carried out by only insiders and 2) acts that outsiders can be carried out without insider assistance. The analysis draws upon historical cases to evaluate the likelihood that malicious acts would be undertaken successfully and to focus on those acts that likely could cause a breach leading to escape of a GoF influenza virus, SARS-CoV, or MERS-CoV. Table 7.4 summarizes the likelihood of an outsider or insider to successfully carry out a particular malicious act and the likelihood that such an act could lead to GoF virus escape.

Table. 7.4. Malicious Acts Undertaken and Likelihood of Success

		Outsider	Insider	Leads to GoF Virus Escape
Armed Assault				
Bomb				Depends of size, type, and location of a bomb blast
Arson				
Physical Entry			N/A	Unlikely by itself
Cyber Breach				
Theft of Virus	Infect Co-Workers			
	Infect Public			
Theft of Animals				
Theft of Materials, Equipment, or Information				
Sabotage				
Elicitation of Information			N/A	
Subversion of Employees			N/A	Unlikely by itself
Insertion of Operative			N/A	Unlikely by itself
Reckless Act				Depends on the act
Deliberate Self-Infection				

Black *indicates consistency of intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Dark ***Grey*** *indicates possible intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Grey *indicates inconsistency of intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Armed Assault

The increase in active shooter incidents in the US suggests that an armed assault in a high containment research laboratory may be possible at some level. Outsiders could carry out an armed assault outside the building in which GoF research is conducted. Insiders possibly could carry out an armed assault inside a research building and high containment research laboratory.

Current personnel security measures requiring periodic assessment of personnel and non-punitive reporting of behavioral changes in personnel could provide an opportunity for institutional officials to identify potential insider threats before acts are conducted. These measures are required for Tier 1 BSAT laboratories. In addition, institutions conduct emergency response exercises and many universities have threat assessment teams to evaluate the threats on campus and identify prevention strategies. Physical security measures, including physical barriers, access controls, and surveillance measures, work to prevent armed outsiders from gaining access to high containment, research laboratories. However, no physical security measures are in place that would prevent authorized insiders from taking guns into high containment research laboratories.

An armed assault leading to escape of a GoF virus is unlikely even if the assault is carried out successfully. Exposure to GoF virus through an open wound is unlikely to cause infection. However, active shooters inside a laboratory might lead to viral escape through accidental aerosolization of virus in experimental samples (i.e., exposing the shooter to aerosolized GoF virus or contaminating street clothing with fomites). If emergency personnel also enter the laboratory, they may be exposed to aerosolized virus or fomites if not wearing proper protection.

Bomb or Arson

Several historical cases exist of malicious actors detonating bombs in public areas, outside buildings, or at clinics or setting fires to research buildings. Although outsiders could detonate bombs outside of buildings or areas accessible to the public, they do not have access to high containment research laboratories unless assisted by an insider. Insiders potentially could detonate bombs inside research buildings or high containment research laboratories.

As with armed assault, current Tier 1 personnel security measures could provide opportunities to prevent insiders from successfully detonating a bomb or setting a fire in a research laboratory and building. Institutions conduct emergency response exercises and universities have threat assessment teams to evaluate the threats on campus and identify prevention strategies. Although physical security measures help prevent outsiders from gaining access to high containment research laboratories, these measures would not prevent authorized insiders from detonating bombs or setting fires in high containment research laboratories.

The size, type, and location of a bomb blast may lead to escape of GoF virus from experimental samples. If the size of the blast is sufficiently large to rattle the building infrastructure (to a similar degree as an earthquake), GoF virus might aerosolize from spilled experimental samples leading to possible loss of containment. Similarly, a blast that occurs at the entrance or inside of a high containment, research laboratory might result in aerosolization of GoF virus and compromise the negative pressure of the laboratory. However, arson is unlikely to lead to escape of a GoF virus even if carried out successfully because institutions have established procedures and response measures for fires and viruses are sensitive to high temperatures.

7.4.1.2.1 Physical Entry

By definition, insiders have authorized access to high containment research laboratories. Consequently, this type of act does not apply to insiders. Outsiders are unlikely to gain physical access to high containment, research laboratories without assistance from an insider.

Physical security measures employed at high containment research laboratories and animal facilities, including physical barriers, access controls, and surveillance measures, help prevent outsiders from gaining access to the laboratory itself.

Physical entry alone does not lead to escape of GoF virus from containment.

Cyber Breach

Over the past decade, a growing number of malicious actors, from nation-states to individuals, have hacked into computer systems in the pharmaceutical, health care, insurance, national security, and commercial organizations. Furthermore in 2010, a computer worm that infected the software of an Iranian uranium enrichment plant, in addition to other industrial sites, affected operations of Iranian nuclear centrifuges,[387,388] suggesting this attack approach should not be ruled out.

Outsiders or insiders with the requisite expertise could hack into the computer systems of research institutions. Other than firewalls, anti-virus software, and standard cyber security measures, no specific measure is required to protect information and infrastructure systems from cyber breaches. The exception is Biological Select Agents and Toxins laboratories, which are required to have information security in place to prevent cyber breaches.[389,390,391,392,393,394]

The likelihood that a cyber breach would lead to escape of GoF virus is moderate. However, breaches in infrastructure systems, such electronic controls for air-handling, could lead to escape pf a GoF virus from containment. That said, air-gapped systems (i.e., those systems that are not connected to the internet) are much less likely to lead to escape of GoF virus. Based on our interviews, systems that control laboratory operations, air filtration, and decontamination are not connected to the open internet, but this does not necessarily mean that the systems are immune to attack.

Theft of GoF Influenza Virus, SARS-CoV, or MERS-CoV

Theft of GoF virus could occur in two ways: 1) by stealing it from a high containment, research laboratory and 2) theft or diversion during transportation. Outsiders acting without the assistance of an insider likely are not able to steal GoF virus from high containment laboratories because of the various access control measures in place to prevent unauthorized access into these laboratories. The likelihood of an outsider stealing GoF virus during transportation is low primarily because knowing the transfer date,

387 Kushner D, The Real Story of Stuxnet. IEEE Spectrum. Accessible at: http://spectrum.ieee.org/telecom/security/the-real-story-of-stuxnet. Accessed on November 4, 2015.

388 Langner R. To Kill a Centrifuge: A Technical Analysis of What Stuxnet's Creators Tried to Achieve. Accessible at http://www.langner.com/en/wp-content/uploads/2013/11/To-kill-a-centrifuge.pdf. Accessed on November 5, 2015.

389 42 C.F.R. §73.11(c)(1).

390 42 C.F.R. §73.11(c)(9).

391 9 C.F.R. §121.11(c)(1).

392 9 C.F.R. §121.11(c)(9).

393 7 C.F.R. §331.11(c)(1).

394 7 C.F.R. §331.11(c)(9).

exact truck carrying the virus, and transportation line used would be extremely difficult without assistance by a knowledgeable insider.

The likelihood of an insider successfully stealing a GoF virus from a laboratory is high because, by definition, such an individual has authorized access to high containment research laboratories. Furthermore, insiders have used pathogens against co-workers, family members, and members of the public in the past. Current personnel security measures requiring periodic assessment and non-punitive reporting mechanisms could identify behavioral changes in personnel before an act is committed. Inventory measures could help identify discrepancies in stored GoF virus. However, theft of virus could occur in-between the regular inventory reviews, could be missed if virus is removed from vials, or could be via theft of experimental, infectious samples.

Theft of GoF virus leads to escape of virus from containment by definition, but such an act does not presume that the stolen virus could be used as a weapon. A significant amount of processing, including growth of the virus from a frozen stock, may have to be carried out to make the virus usable and/or disseminable. However, theft of experimental samples that contain GoF viruses might be used as is.

Theft of Animals

Several historical examples exist of domestic animal rights extremists removing animals from low containment research laboratories to take home as pets or release into the wild. However, no examples exist for high containment research laboratories or animal housing facilities, likely because of increased security and access controls of both facilities. The physical security and perimeter surveillance measures of facilities where research animals are present have increased to counter deliberate acts carried out by animal rights extremists. Current personnel security measures involving periodic assessment (as in Tier 1 BSAT) and non-punitive reporting mechanisms might identify insiders who have been elicited, recruited, or subverted by outsiders or decided to carry out a malicious act on his/her own. Vigilance by other laboratory workers could decrease the likelihood that animals go missing.

Theft of infected animals would likely lead to escape of GoF virus.

Theft of Materials, Equipment, and Information

Despite numerous historical cases involving deliberate acts carried out by domestic terrorists and extremists, none have involved high containment research laboratories. The historical cases evaluated involved assistance from insiders to provide information, enable access into laboratories, or carry out actual deliberate acts, such as theft of animals or vandalism of equipment. Physical barriers and access control measures prevent outsiders from gaining access to high containment research laboratories. Therefore, outsiders acting without insider-assistance are unlikely able to steal laboratory materials, equipment, and information contained in high containment research laboratories. Because insiders are authorized to access high containment research laboratories, the likelihood that they could steal materials, information, or equipment is high.

Current personnel security measures involving periodic assessment (as in Tier 1 BSAT) and non-punitive reporting mechanisms might identify insiders who have been elicited, recruited, or subverted by outsiders or decided to carry out a malicious act on his/her own. Vigilance by other laboratory workers could decrease the likelihood that equipment, materials, or information (e.g., laboratory notebooks or inventory logs) go missing.

Theft of contaminated equipment or materials might lead to escape of GoF virus. Theft of information would not lead to escape of GoF virus.

Sabotage

The likelihood that outsiders will tamper with equipment or experiments in high containment is low if not assisted by an insider. The likelihood that an outsider will tamper with the laboratory itself (including the HEPA filtration system and waste management system) is low unless such an individual has assistance from a knowledgeable insider. An insider with access to experiments and equipment could tamper with them. However, not all insiders have access to laboratory operating systems, reducing the likelihood of such acts.

Current physical barriers and access controls help prevent outsiders from gaining access to high containment, research laboratories, and their primary operating systems. Personnel security measures help identify insiders who might carry out acts of sabotage within the laboratory, against a facility, or in the laboratory operating system. However, these measures would not necessarily enable detection of insiders in a non-BSAT high containment research laboratory.

Sabotage of experiments, equipment, or laboratory operating systems might lead to escape of GoF virus. For example, tampering with laboratory materials could lead to ineffective decontamination of samples, which, if undetected, could result in accidental exposure of laboratory workers who don't realize the viral samples are still infectious. Other examples include removal of HEPA filters from the air flow system, which would prevent proper filtration of the laboratory air, or tampering with a centrifuge rotor, which could result in an imbalance during spins causing the contents to rupture and exposing laboratory workers to the infectious samples.

Reckless Act

Reckless acts include mixing of infected animals with uninfected animals to deliberately tamper with experiments. Several historical cases involving animal rights extremists suggest that these acts can be carried out in low containment research laboratories. However, the increase in physical barriers, access control measures, surveillance of animal facilities, and arrests have deterred such groups from carrying out these acts.

These types of reckless acts are highly implausible for GoF virus research, because infected and uninfected experimental animals are kept in high containment research laboratories, which often are in different locations than facilities housing uninfected animals that are not part of ongoing research. Consequently, the likelihood that an outsider could carry out such acts is low unless assisted by an insider. The likelihood that insiders who have been elicited, recruited, or sabotaged by an outside group could remove animals from high containment, research facilities is high. However, because animals involved in active experiments are separated physically from animals not involved in experiments suggests that mixing of infected and uninfected animals in lower containment is not as likely, though not impossible.

Physical barriers, access control measures, and surveillance of animal facilities and BSAT facilities help prevent outsiders from entering high containment, research laboratories unassisted. Current personnel reliability measures, including periodic assessment and non-punitive reporting mechanisms, help institutional officials to detect changes in behavior in personnel. However, these personnel security measures are required only for Tier 1 BSAT laboratories; some non-Tier 1 BSAT laboratories implement these measures on their own or as part of their institution's Tier 1 BSAT program, if applicable.

Reckless acts, such as removal of experimental animals, could lead to escape of a GoF virus via the infected animal. Mixing of infected and uninfected animals could lead to escape of a GoF viruses if the

uninfected animals are in low containment and in contact with people. Deliberate infection of oneself, co-worker, friend, or family member leads to escape of a GoF virus.

Deliberate Self-Infection

Two historical cases of deliberate self-infection exist; however, these cases do not involve self-infection with a virus taken from a research laboratory. Acts involving deliberate self-infection require an actor to obtain the GoF virus either from a high containment research laboratory. Outsiders acting without the assistance of an insider are not likely to obtain a GoF virus from high containment laboratories because of the various physical barriers and access controls in place at such laboratories. The likelihood of an outsider obtaining GoF virus during transportation is low primarily because knowing the transfer date, exact truck carrying the virus, and transportation line used is impossible without assistance by a knowledgeable insider.

The likelihood of an insider obtaining a GoF pathogen from the laboratory for use in self-infection is high because (s)he has authorized access to high containment research laboratories. Current personnel security measures requiring periodic assessment and non-punitive reporting mechanisms could identify behavioral changes in personnel before an act is committed. The benefit of inventory measures for acquisition of virus for self-infection is unclear. The insider likely would use experimental samples, which are not part of current long-term storage measures.

Deliberate self-infection of insiders would lead to escape of GoF virus from containment.

7.4.1.2.2 Malicious Act Conclusion

The most likely malicious acts that could lead to escape of a GoF virus from high containment research laboratories are: theft of GoF virus or contaminated equipment; tampering with experiments or laboratory operating systems; removal of infected animals; and deliberate infection of oneself, friend, family member, or co-worker. All of these acts involve insiders, who are either acting alone or in coordination with a group, such as domestic terrorist and extremist group. Whether theft of GoF virus leads to exposure and infection by laboratory workers or members of the public depends on the virus' form (either from frozen vials or experimental samples) and the skills and resources of the malicious actor to effectively grow and deliver the virus.

A possible malicious act that is less likely to lead to escape of a GoF virus is a bomb. The size and location of a bomb determines whether its detonation could lead to escape of GoF virus in experimental samples. Outsiders and insiders could detonate a bomb, though in different locations (i.e., outside the building or near a high containment, research laboratory).

7.4.1.3 Type of Breach Leading to GoF Virus Escape

The likelihood of virus escape and human infection caused by malicious acts are summarized in Table 7.5.

Table 7.5. Type of Breach Leading to Virus Escape

		Malicious Act	GoF Virus Escape	Human Infection
Loss of Containment	**Release of Infected Animals from and within Laboratories**	Theft of Animals Sabotage Reckless Act		
	Release of Infected Animals in the Environment	Theft of Animals Reckless Act		
	Cross-Contamination of Laboratory Animals	Sabotage Reckless Act		
	Exposure of Laboratory Workers (Could Include Emergency Personnel accessing the Laboratory)	Armed Assault Bomb Sabotage Reckless Act Deliberate Self-Infection		
	Removal of GoF Virus from the Laboratory	Theft of GoF Virus		
Deliberate Outdoor Release of GoF Virus	**Infection of Wild or Domestic Animals**	Theft of GoF Virus Theft of Animals		
	Infection of Laboratory Workers	Theft of GoF Virus		
	Infection of the Public	Theft of GoF Virus Theft of Materials Theft of Equipment		

Black *indicates consistency of intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Dark ***Grey*** *indicates possible intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Grey *indicates inconsistency of intent, capable, or able to access high containment, research laboratories with known malicious actor motivations and capabilities, and historical incidents.*

Release of Infected Animals From and Within Laboratories.

The design of and security measures associated with high containment research laboratories help prevent unassisted escape of animals from the laboratory. However, if an insider intentionally releases laboratory animals outside of high containment research laboratories, the likelihood that animals will escape the building varies based on the number of released animals, the method of release, their ease of capture by researchers, and the design features of the facility, all of which either limits or permits animal escape. Furthermore, the number of people that the animal might encounter as it wanders around in the building affects the level of exposure these individuals have to GoF virus from infected animals. Because of this variability, the likelihood of GoF virus escape and human infection resulting from theft of infected animals from and within laboratories is moderate and depends on a variety of factors.

Release of Infected Animals in the Environment

Theft of animals would result deliberate release of infected animals into the environment, whether in the wild or someone's home (as a pet), and hence, would be considered as a GoF virus escape. Furthermore, the close proximity of the infected animal to the actor who releases the animal suggests that at least one human (the malicious actor) would be exposed to the GoF virus and could be infected.

Cross-Contamination of Laboratory Animals

Sabotage of experiments, including the deliberate mixing of infected and uninfected animals within high containment research laboratories, neither increases the likelihood of GoF virus escape, nor increases the likelihood of human infection. However, the deliberate mixing of infected and uninfected animals in lower containment research environments (i.e., sabotage) might expose researchers not protected against H5 influenza, SARS-CoV, or MERS-CoV to GoF virus and cause GoF virus escape if the virus gets on street clothing. The likelihood that this exposure could result in human infection depends on the level of exposure researchers have with the infected animals before the contamination is detected.

Exposure of Laboratory Workers.

Malicious acts involving deliberate or accidental exposure of laboratory workers could result in GoF virus escape if the exposed individual(s) gets infected with the GoF virus. Human infection may occur with virus from experimental samples, thawed virus, or fomites. If equipment or other materials are deliberately contaminated or tampered with and laboratory workers are not protected well (i.e., through use of the appropriate personal protective equipment), they may get infected with GoF virus in experimental samples. Consequently, the likelihood of human infection is moderate.

Removal of GoF Virus from the Laboratory.

By definition, theft of GoF virus from the laboratory results in viral escape. However, the degree to which GoF virus removal causes human infections depends on the form of the virus (i.e., either frozen virus or virus in experimental samples) and/or the skills, expertise, and resources of the malicious actor to grow or manipulate frozen viruses. Consequently, the likelihood of human infection is moderate.

Infection of Wild or Domestic Animals Following Deliberate Outdoor Release of GoF Virus.

By definition, release of stolen GoF virus from experimental samples, stocks grown from stolen virus, or stolen infected animals into the wild or households results in viral escape. The likelihood that a malicious insider would be able to make a sophisticated dispersal device and not be detected is low, suggesting that rudimentary dispersal devices may be the most likely route of release of virus. Furthermore, the likelihood that infected animals (domestic or wild) could cause immediate infection in humans is low because of the low level of interaction between wild animals and humans or domestic animals in urban settings. However, the zoonotic nature of the viruses (i.e., their ability to infect animals and at least some humans) does not automatically rule out the possibility of human infection ever.

Infection of Laboratory Workers or the Public following Deliberate Outdoor Release of GoF Virus

Exposure of laboratory workers or members of the public using stolen GoF virus from experimental samples or stocks grown from stolen virus results in GoF virus escape and human infection.

7.4.1.3.1 Type of Breach Conclusion

The most likely types of breach leading to human infection following a malicious act are release of infected animals, infection of laboratory workers following deliberate release of GoF virus, and infection of the public following deliberate release of GoF virus. However, successful release depends on form of the virus (i.e., frozen stock or experimental sample) and the skill-level of malicious actors (to grow virus from frozen stock). These breaches could only occur with the assistance of an insider or significant blast that affects the integrity of the laboratory.

Given the right circumstances, human infections might occur from release of infected animals from and within laboratories, cross-contamination of laboratory animals, exposure of laboratory workers, and removal of GoF virus from the laboratory. The number of people exposed in each of these cases is likely to be low, suggesting an even lower rate of infection among exposed individuals.[395]

7.4.1.4 Plausible Threats of GoF Viruses

The most plausible threats facing laboratories in which GoF virus research is stored or studies are those carried out by insiders, acting alone or in cooperation with a domestic terrorist group or extremist group. Insiders acting alone may be disgruntled, emotionally disturbed, or radicalized. Those cooperating with a group may be sympathetic to the group's cause, coerced, or subverted.

Most likely, insiders will commit acts covertly. Such acts would most likely expose a small number of people to GoF virus. If exposed individuals are familiar with the symptoms and disease progression of the viruses, they might seek help immediately if infected. If not (i.e., the general public), infections resulting from exposure could lead to secondary infections. In addition, insiders could use GoF virus to expose a large number of people.

Though less plausible, insiders might commit overt acts, such as arson, bombing, or armed assault. Some of these acts would not lead to GoF virus escape and human exposure. The assumption is that emergency responders and public health officials will respond quickly to overt acts involve active shooters, fire, or explosions.

Most acts involving malicious actors without insider-assistance are not plausible. However, outsiders, including transnational terrorists, domestic extremists, domestic terrorists, and lone outsiders, could carry out an armed assault or detonate a bomb at the building perimeter if they have access. Armed assault would not lead to GoF viral escape and human exposure. However, a bomb of sufficient size might affect laboratory operating systems, possibly leading to release of GoF virus from experimental samples. These acts are overt and would elicit response from emergency responders.

Table 7.6 summarizes the results of the analysis. These results provide the basis for epidemiological modeling of plausible security threats involving GoF virus.

[395] No known virus has 100% infection rate among exposed individuals.

Table 7.6. Plausible Threats Involving High Containment Research Laboratories That Store or Study GoF Viruses		
Overt	Insider	Active shooter or physical assault Bomb detonated near or inside high containment space
	Outsider	Bomb detonated at building periphery
Covert Act (Expose Public)	Insider	Removal of GoF virus (frozen stock or experimental sample), infected animals, or contaminated equipment
Covert Act (Expose Laboratory Workers)	Insider	Removal of GoF virus in experimental samples Deliberate contamination of personal protective equipment or laboratory equipment Deliberate compromise of laboratory equipment or personal protective equipment Mixing of experimental samples or animals into lower containment

In addition to these plausible threats, theft of information about research, facilities, hours of operation, and personnel records are likely by foreign intelligence or domestic extremist groups.

7.4.2 Semi-Quantitative Epidemiological Modeling of Security Risks

7.4.2.1.1 The Need for a Semi-Quantitative Approach

The section above identified malicious acts that could plausibly be caused by a malicious actor and lead to a loss of containment event. The variability in the manner through which these malicious acts could be executed and the unknown probabilities of success at each step precludes the designing of fault trees (as was done for accidents in the Biosafety Risk Assessment) for these malicious acts. That is, no evidence-based quantitative model can be designed to estimate the probability that a particular malicious event would be successful the amount of virus escaping containment from a successful malicious act. Moreover, the state of the threat information is such that even estimating the frequency with which these malicious events would be attempted would prevent open and transparent communication of the risks. For this reason, a semi-quantitative approach is leveraged that estimates the difference in consequences between a malicious act targeting a laboratory with wild type strains vs one targeting a laboratory with various GoF strains, assuming that the malicious act were successful in causing at least one initial infection. This section culminates with an estimate of the frequency with which these malicious acts must be successful for the biosecurity risk to approximate the biosafety risk (given the relative consequences of the two types of events). Throughout, the consequences computed from various events in Chapter 6- Biosafety Risk are used where appropriate.

In most cases, any GoF trait would increase risk by either increasing the chance that an outbreak, initiated by an infection caused by a malicious event, would escape local control to seed a global outbreak, or by increasing the consequences of a global outbreak. Two GoF traits theoretically could influence the chance that an initial infection outside the laboratory would occur due to the malicious act: 1) enhanced growth in culture (increasing the amount of contamination that could escape the laboratory) and 2) adaptation of avian influenza strains to mammals so that the median infectious dose is decreased.

The first part of this section evaluates the potential for these two phenotypes to influence the probability that an initial infection occurs. The sections that follow discuss how all other GoF phenotypes could affect risk of an outbreak should an initial infection occur.

7.4.2.2 Influence of GoF Traits on the Probability That an Infection Outside the Laboratory Would Occur from a Malicious Act

The phenotypes of enhanced viral growth in culture and adaptation to mammals have the potential to increase infection probability in loss-of-containment incidents. Of all of the pathogens assessed in this study, this section is relevant only to influenza. Coronaviruses are already adapted to human hosts and so this phenotype is meaningless for these pathogens. Moreover, coronaviruses already grow to high titers and for this reason, no GoF manipulation is necessary to enhance their growth. (In any case, should a scientist attempt to enhance their growth or decrease their infectious dose in people, the analysis herein would suggest that little biosecurity would inhere in these manipulations.)

Figure 7.6 explores the relationship of the amount of contamination released (which is influenced by the titer of the sample leading to the contamination) in two strains of influenza, one with a relatively high median infectious dose (like avian influenza—top panel) and one with a very low infectious dose (of 1.5pfu—bottom panel). Increasing the amount of pathogen escaping the laboratory by an order of magnitude increases the probability of at least one infection by roughly 10%. That is, if a strain grew to a 100-fold greater titer due to a GoF manipulation and that concentrated stock caused contamination that left the laboratory in a malicious act, the act would have only a 20% increase in the chance that an infection would occur. Unless a very little amount of contamination leaves the laboratory (less than 100 pfu), this increase in the contamination released would increase the overall chance that an infection occurs by less than a factor of two. Moreover, the analysis shown in Figure 7.6, assumes that an enhancement of viral growth leads to a similar increase in the contamination released. In reality, viruses that grow to a high titer are diluted for use in most experiments (such as plaque assays or challenge experiments) so only some cultures that could cause contamination would be at the greater concentration enabled by a GoF experiment.

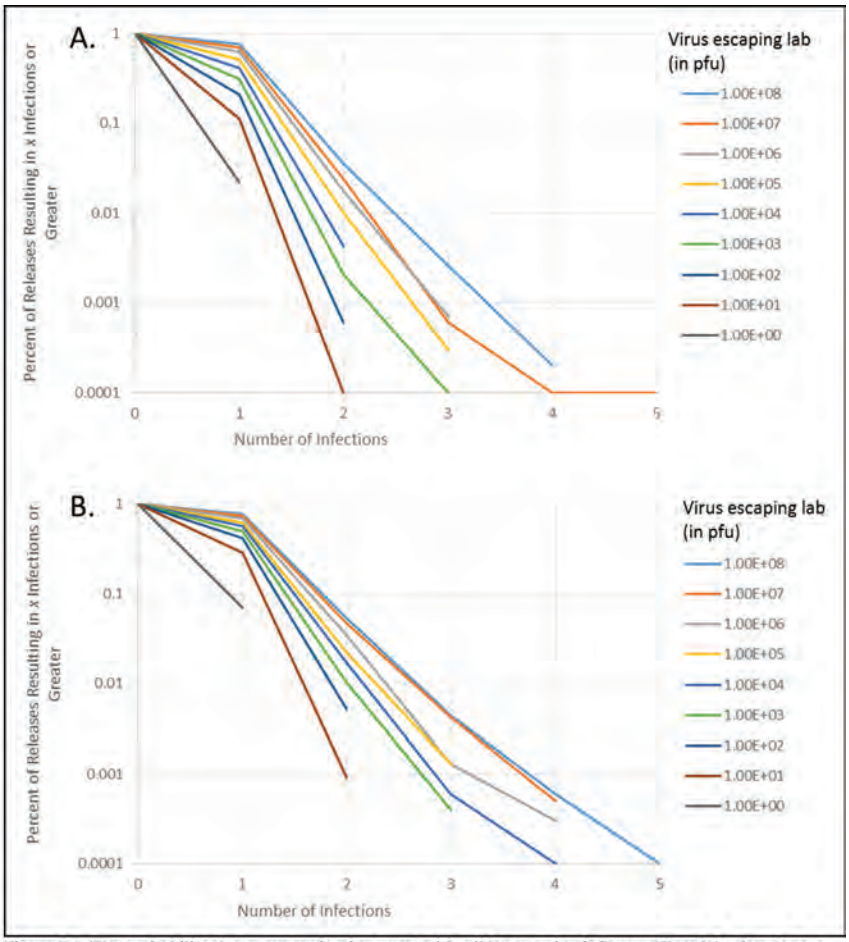

Figure 7.6. The probability that a contamination event with wild type avian influenza (Panel A, above), or a modified strain with a median infectious dose of 1.5pfu (Panel B, below) causes a certain number of infections when contaminated material escapes the laboratory for a variety of viral loads. The y-axis shows probability on a log scale with 1=100% certainty.

Comparing the panels in Figure 7.6. shows that the adaptation of avian influenza strains to mammals (resulting in a lower median infectious dose) would increase the probability that at least one person was infected by the contamination event by a factor of two or three. If just one pfu contaminates someone leaving the laboratory, one person would be infected about 7% of the time if the strain had a low infectious dose, compared to 2% if the strain were not adapted to humans. If the contamination involved

100 pfu, the chance of at least one infection would increase from 20% to 40% if the infectious dose were to decrease. However, strains adapted to humans are likely to be minimally infectious to birds and therefore malicious acts that involve the contamination of wild life (such as the release of infected birds) would be a lower total risk if this manipulation occurred. If the avian influenza strain were not also manipulated to be transmissible among humans, a malicious act resulting in the escape of contamination would sicken at most five people (Figure 7.6). In contrast, a strain that is not adapted to humans could cause an outbreak in avian species (if an infected animal were released, for example), which would lead to much more severe human health consequences (up to 1,000 illnesses and 100 deaths) than the direct infection of laboratory workers or the public by contamination. Recall that the biosafety risk assessment estimated that adaptation of an avian strain to humans (without increasing transmissibility) would *decrease* risk by a few fold because accidents that lead to an avian outbreak are much more likely than those that infect a person and avian outbreaks could lead to the deaths of many people, not just a single person infected in a laboratory. For this reason, because biosecurity events could lead to the infection of people or wildlife, we presume that adaptation to humans neither significantly increases nor decreases biosecurity risk.

Events that involve the release of contamination from the laboratory could also result in the infection of birds, although that chance is remote. Specifically, even assuming that 1E8 pfu of avian influenza escapes the lab on a single person's hand, the fomite model predicts that no infections would occur in chickens, ducks, or turkeys in 300,000 simulations. This result is not surprising because of the short half-life of influenza on the skin (on the order of minutes) and the rarity of laboratory workers physically handling poultry outside of a laboratory. No GoF phenotype would make the infection of birds from such a contamination event more likely or more extensive should it occur because wild type strains of avian influenza are already highly contagious and highly pathogenic in birds. The human health consequences from such an outbreak are estimated to involve 100 deaths and 1,000 illnesses.

7.4.2.3 Aligning the Malicious Acts to Biosafety Scenarios to Calculate Risk of Wild Type Agents

The probability that a malicious incident results in an outbreak that escapes local control depends heavily on who is initially infected (a laboratory worker or a member of the public), if the infection is related to an overt or covert incident and, to a lesser degree, how many people were initially infected. Events that initially target laboratory workers are of lesser risk than those that target the public because laboratory workers are more likely to be vaccinated against the strains in their laboratory, to self-monitor for initial signs of illness (like a fever), and to be isolated should an unusual illness manifest. If the event is overt and poses a high risk of causing an infection, the laboratory worker (or any person responding to an event in a laboratory) could be given antivirals prophylactically and also would be more closely monitored for the initial signs of illness (and could even be preemptively isolated).

Simply put, because of these four critical parameters, all malicious acts can be grouped into four categories to consider the risk of a global outbreak of a human transmissible disease: overt infections of laboratory workers, overt infections of members of the public, covert infections of laboratory workers, and covert infections of members of the public (Figure 7.7). For diseases that can spread amongst wildlife but not people (specifically, wild type avian influenza), a fifth group, malicious acts specifically infecting wildlife, is considered.

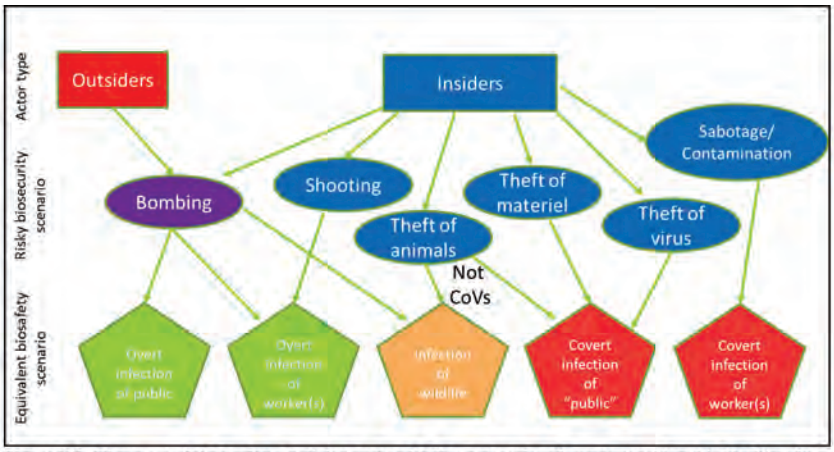

Figure 7.7. Alignment of the various malicious acts with five categories of events considered in the biosafety risk assessment. Because coronavirus strains that are modified to infect other animals pose a limited risk to people, we do not consider theft of animals is likely to cause an outbreak of human-transmissible disease.

7.4.2.4 Probability That a Malicious Event Would Lead to a Global Outbreak

From the Biosafety Risk Assessment in Chapter 6, we can determine the probability that an outbreak, caused by a malicious event, would lead to a global outbreak (Table 7.7). The table shows the probability that at least one secondary case would be caused and the probability that the outbreak would escape local control if laboratorians were infected or if members of the public were infected. Clearly, these probabilities are greatly influenced by how many people were infected by the initial event. Table 7.7 shows how these probabilities change if just one person were initially infected or several people were initially infected by the event. We assume that only five people in a laboratory could be simultaneously infected by an event due to the relatively small numbers of people working in a containment suite at any given time.

Table 7.7. Probability That the Initial Cases Lead to Secondary Infections, and the Probability That the Outbreak Escapes Local Control for Each Type of Event

Event Type		Seasonal Influenza		Pandemic Influenza		Avian Influenza		Coronaviruses	
Risk Type	**# of Initial Cases**	**Prob. of secondary spread**	**Prob. escapes local control**	**Prob. of secondary spread**	**Prob. escapes local control**	**Prob. of secondary spread**	**Prob. escapes local control**	**Prob. of secondary spread**	**Prob. escapes local control**
Infection of wild life		N/A	N/A	N/A	N/A	100%	100%	N/A	N/A
Covert infection of public	1	55%	20%	60%	20%	0%	0%	35%	0%
	10	100%	90%	100%	95%	0%	0%	100%	0%
Covert infection of worker(s)	1	15%	20%	20%	20%	0%	0%	2%	0%
	5	85%	90%	85%	95%	0%	0%	32%	0%
Overt infection of public	1	30%	20%	40%	20%	0%	0%	25%	0%
	10	100%	90%	100%	90%	0%	0%	100%	0%
Overt infection of workers	1	1%	20%	1%	20%	0%	0%	1%	0%
	5	3%	90%	3%	90%	0%	0%	5%	0%

Events involving wild type viruses highlighted in yellow have a relatively low probability of causing a pandemic and therefore offer an opportunity for GoF manipulations to increase risk.

As can be seen from the table, events that lead to at least one infection in members of the public are five- to 30-fold more likely to initiate a local outbreak than those that infect laboratory workers. Events that covertly infect laboratory workers are 10- to 20-fold more likely to initiate a local outbreak than those that overtly infect laboratory workers. This analysis permits a relative ranking of the risk (assuming the probabilities of the relative chance of success and the frequency of the malicious acts are unknowable), which is shown in Table 7.8.

Table 7.8. Relative Risk of the Plausible Malicious Acts Given an Unknown Frequency of Occurrence and Probability of Success

Risk Category	Primary Infection	Overt vs Covert	Event
Highest	Public or Wildlife	Covert	Theft of animals
Highest	Public	Covert	Theft of equipment, theft of virus
Moderately High	Public or Wildlife	Overt	Bombing
Moderately Low	Laboratorians	Covert	All events that lead to the infection of co-workers directly or indirectly
Lowest	Laboratorians	Overt	Shooting

Events that lead to an infection of wildlife are relatively low risk because those cannot seed a global pandemic of a human transmissible disease.

The potential for GoF phenotypes to increase the probability that a global outbreak occurs following an infection initiated by a biosecurity event is taken directly from the Biosafety Risk Assessment (Chapter 6 and summarized in the Stop Light Chart shown in Figure 7.8). This figure shows that transmissibility is the trait that can most affect the probability that the outbreak would escape local control, and this statement holds true for all pathogens evaluated. For seasonal and pandemic influenza, the ability to evade residual immunity or an increase in transmissibility to that of newly emergent pandemic influenza strains would increase the probability of a global outbreak. The relatively low risk that an infected laboratorian would infect another person is due to robust health monitoring and isolation protocols. GoF traits do not reduce the ability of these measures to mitigate an incident. If the strain were more pathogenic, perhaps the public fear elicited would improve social distancing measures and *decrease* the probability that an outbreak is contained, but this possibility cannot be directly evaluated. The ability to overcome protective vaccination and antiviral resistance independently modestly increases the chance that an infected laboratory worker would cause a secondary infection, so this trait has minimal influence on risk. Lastly, no explicit plans exist for the extensive use of antivirals in an outbreak associated with a laboratory, so the role of antivirals in a nascent outbreak could not be determined.

GoF Phenotype	Seasonal Influenza Viruses	Pandemic Influenza Viruses	Avian Influenza Viruses	Coronaviruses
Enhanced transmissibility				
Enhanced pathogenicity	Unknown	Unknown		
Adaptation to mammals	N/A	N/A		N/A
Evasion of induced immunity			N/A	N/A
Evasion of natural/residual immunity			N/A	N/A
Antiviral resistance				N/A
Enhanced growth in culture/eggs	N/A	N/A	N/A	N/A

Figure 7.8. A chart showing the relative increase in the probability that a global outbreak would occur for a variety of pathogens with GoF traits compared to the same strains with wild type traits. Darker grey denotes increasing risk. Green indicates that the phenotype does not increase risk for that pathogen.

Since wild type avian strains are not transmissible among people, the hazard ends with those initially infected by the event unless wild birds are infected (causing a global avian outbreak) or the strain is modified to transmit among humans. If the strain were modified to be as transmissible in humans as seasonal or pandemic influenza, the risk of a global outbreak would be significant. For this reason, GoF studies that increase the transmissibility of avian strains in humans significantly increase the probability that a global outbreak would occur. No other GoF traits affect the probability that an outbreak seeds a global pandemic for avian influenza.

Similarly, the coronaviruses are insufficiently transmissible to have a significant chance of seeding a global pandemic. Strains with enhanced transmissibility increase the chance that an outbreak occurs and that this outbreak sparks a global pandemic.

7.4.2.5 The Influence of GoF on the Consequences of a Global Pandemic

Should a global outbreak be sparked by a malicious act targeting a laboratory, the consequences would be similar to a global outbreak sparked by an accident in a laboratory and the influence of GoF traits on risk would be identical to those explored in Chapter 6. Figure 7.9 summarizes those findings.

GoF Phenotype	Seasonal Influenza Viruses	Pandemic Influenza Viruses	Avian Influenza Viruses	Coronaviruses
Enhanced transmissibility				
Enhanced pathogenicity				
Adaptation to mammals	N/A	N/A	N/A	N/A
Evasion of induced immunity			N/A	N/A
Evasion of natural/residual immunity			N/A	N/A
Antiviral resistance				N/A
Enhanced growth in culture/eggs	N/A	N/A	N/A	N/A

Figure 7.9. A chart showing the relative increase in the probability that a global outbreak would occur for a variety of pathogens with GoF traits compared to the same strains with wild type traits. Darker grey denotes increasing risk. Green indicates that the phenotype does not increase risk for that pathogen.

For seasonal and pandemic influenza, antiviral resistance and the ability to overcome protective vaccination would not significantly increase deaths from an outbreak globally, but would increase deaths by a few fold in North America due to the availability of these countermeasures in the US. Note, however, that to effectively evade the protection afforded by a vaccine raised in response to a particular outbreak, the strain must be modified to overcome vaccination regardless of its antigenic profile, which is not a subject of active GoF research. Increasing the transmissibility (or, similarly, imbuing the ability to evade residual immunity) of seasonal or pandemic influenza can increase global deaths. Given the relatively low case fatality rate of seasonal influenza, significant increases in pathogenicity (10x or more) are possible and these would proportionally increase the death toll.

A wild type avian influenza strain can infect people only via contact with infected birds, resulting in a few thousand cases at best. Given that many strains are minimally pathogenic, increasing the pathogenicity in people could increase these deaths by a few fold. In contrast a strain modified to be transmissible in people could cause a global outbreak, infecting millions and therefore significantly increasing risk. Increasing pathogenicity could increase global deaths by a few fold.

The wild type versions of the coronaviruses are insufficiently transmissible to have a significant probability of causing a global outbreak, or, if they do, the consequences are relatively small. Increasing transmissibility of these strains

7.4.2.6 Overall Influence of GoF on Risk of Biosecurity Events

In summary, only a handful of GoF traits significantly increase biosecurity risk after a malicious event targets a laboratory. For seasonal and pandemic influenza, the ability to overcome protective vaccination and antiviral resistance modestly increases risk by increasing the potential consequences in North America. No significant effect on risk exists if the global population is considered as a whole. Increasing the transmissibility and ability to evade residual immunity significantly increases risk because outbreaks are more likely to occur, escape local control, and create more consequential global outbreaks.

For avian influenza, increasing transmissibility greatly increases risk because this modification is required to spark a global outbreak of a disease by human-to-human contact, potentially infecting millions. Without this change, the hazard is restricted to those exposed to contaminated materials and infected birds, limiting the outbreak to thousands of cases at most. Increasing pathogenicity can modestly increase risk.

Similarly, the wild type coronaviruses have a very small chance of sparking a global outbreak so increasing transmissibility greatly increases risk. Increasing pathogenicity can modestly increase risk.

7.4.2.7 Comparison of Risk of Biosecurity Events Versus Biosafety Events

To understand the biosecurity risk of acts targeting a GoF laboratory relative to the risk of accidents with the same pathogens, this section provides data on the approximate frequency that various malicious acts must successfully result in an infection to match the risk of an *accident* involving the same pathogen. To accomplish this, estimates of the probability that a laboratory acquired infection sparks a global pandemic from the Biosafety Risk Assessment in Chapter 6 are combined with historical rates of laboratory acquired infections. Figure 7.10. shows the return frequency of a laboratory acquired infection in any one of the approximately 100 laboratories that study influenza or the coronaviruses in the US given that no laboratory infections have occurred in the last 20 years (or assuming that a few have occurred that we have not identified).

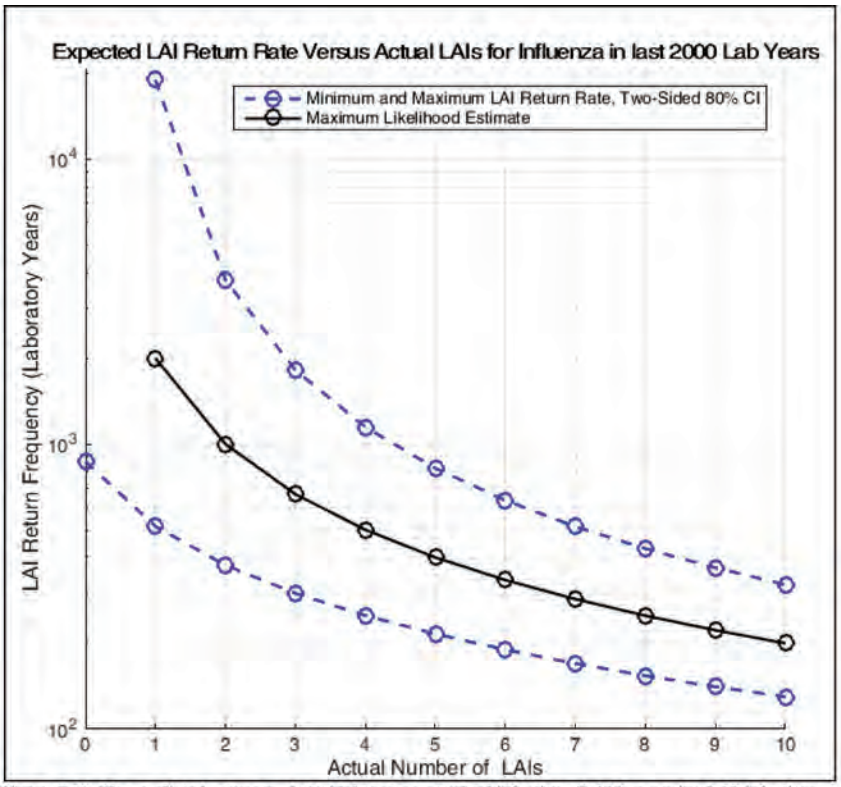

Figure 7.10. The predicted return period of laboratory acquired infections (LAIs) assuming 0-10 infections have actually occurred in the last 20 years across 100 laboratories. The 90th percentile of the maximum rate (bottom line) was used to produce an estimate of the return period that would greater than 90 out of 100 estimates of the frequency, whereas the maximum likelihood estimate and 90th percentile of the minimum rate (top line) is also shown.

A laboratory acquired infection is expected to occur every three to 200 years across all laboratories in the US. For simplicity, all these infections are assumed to be in laboratories that study seasonal influenza, since these vastly outnumber the laboratories that study other pathogens, and this work can be done at BSL-2, which allows more laboratory acquired infections to occur compared to BSL-3. (Highly pathogenic avian influenza, SARS-CoV, and MERS-CoV are studied in BSL-3 laboratories suggesting that the calculated number represents the upper bound of laboratory acquired infections for these agents.)

As described in the Biosafety Risk Assessment (Chapter 6), only about 0.5% of these laboratory infections are predicted to cause global pandemics due to public health response measures, stochastic factors, health monitoring, and isolation protocols. For this reason, a global pandemic due to a laboratory accident is expected to occur every 750-50,000 years.

Given that the highest risk biosecurity events (theft of animals, materials, or stocks by an insider) are also among the most plausible and that these events lead to covert infections of the public, the chance that a biosecurity event that infects one person leads to a global pandemic is much greater than the chance of an accidental laboratory acquired infection (since these may be overt and nearly always infect laboratory workers). If an infection occurs, biosecurity events have an 11% chance of starting a global pandemic (55% chance of initiating an outbreak and a 20% chance that this outbreak escapes local control).

For a biosecurity event to have the same total risk as biosafety events, a successful event that covertly infects the public (theft from an influenza laboratory of an infected animal, contaminated piece of equipment, or viral stock) must occur once every 80-5,500 years (11% of 750-50,000). Given the frequency with which thefts have been perpetrated by insiders in laboratories, this analysis suggests that biosecurity considerations be given as much weight as biosafety issues.

8 Biosecurity Risk of GoF Information

8.1 Summary

In this section, we analyze the risk that a malicious actor might misuse the information in publications describing GoF research. This analysis is based on the open-source literature covering desirable characteristics of biological agents and the scientific literature on GoF studies and non-GoF studies with significant dual-utility. We employed the NSABB definition of GoF research to delineate the dual-use phenotypes considered.[396]

We assessed the potential biosecurity information risk that could be generated by GoF information compared to what could be achieved through dual-use studies that do not rely on GoF research. We then assessed whether the unique dual-use information resulting from GoF studies had already been published. We find that little information risk remains from GoF research (see Figure 8.1). Although the development of a highly-contagious, highly virulent strain of influenza presents significant biosecurity information risk, the methods to produce these strains have already been published and so no information risk remains. Moreover, the specific changes in the genome that led to these traits have also been characterized and published, so an actor could reproduce the dual-use strains using reverse genetics. Although several potentially dual-use studies have already been published, translating animal studies of transmissibility to empirically predict an exact R_0 in a human outbreak is currently impossible; therefore, we cannot determine if the studies already published could be used to create strains of influenza that could cause a global pandemic (R_0 of greater than one). If not, further studies on this topic could create an information risk.

Similarly, information on how to develop strains of influenza viruses that grow well in culture/eggs or evade medical countermeasures or diagnostics has some dual-utility, but the methods to create these strains also have already been published.

[396] Framework for Conducting Risk and Benefit Assessment of Gain-of-Function Research: Recommendations of the National Advisory Board for Biosecurity. May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

Dual-Use GoF Phenotype	Seasonal/Pandemic Influenza	Coronaviruses
Enhanced transmissibility in mammals		
Enhanced pathogenicity in mammals	Published methods require skills in molecular biology or were in poor animal models of pathogenicity. No publications exist on creation of influenza strains that lead to chronic illness.	
Enhanced transmissibility while maintaining pathogenicity		
Overcoming natural or induced immunity	Via the creation of antigenically distinct strains only	N/A
Evading diagnostics		The evasion of diagnostics that target the genomic sequence of the virus may pose an information risk.
Antiviral resistance		N/A
Enhanced production in cell culture or eggs		N/A

Figure 8.1. Summary of the information risk posed by GoF research in influenza (middle set of columns) and the coronaviruses (right set of columns). Information that has a significant dual-use (from Figure 8.2) AND is not yet published (Figure 8.3) is shaded darkly because it poses a remaining information risk. Information that is not actually dual-use OR has already been published is left white because it poses no remaining information risk. Information shaded gray may have some remaining information risk under some circumstances. N/A denotes traits that are not applicable to the coronaviruses.

Significant information risk would be realized by the publication of methods to create a highly transmissible SARS- or MERS-coronavirus that maintains its pathogenicity. Notably, without an animal model of transmissibility for these pathogens, this information risk is unlikely to be realized in the near future. A modest information risk inheres in methods to manipulate the genomic targets of a diagnostic assay for coronavirus infections without compromising the other desirable traits of the pathogen.

A modest information risk would be realized if researchers published methods to produce strains of influenza viruses that can produce more prolonged or chronic illness. Although this manipulation is a possible enhancement of pathogenicity that can fall under the definition of GoF research, there is little scientific rationale to undertake these experiments. Hence, the possibility that this information risk will be realized is low. Another modest information risk inheres in the publication of methods to produce strains of influenza virus that are able to overcome protective vaccination even if the vaccine matches the serotype of the pathogen. Similar work has been published for other pathogens, but these pathogens have larger and more plastic genomes than the influenza viruses so it is not known if similar manipulations could be successfully carried out in the influenza viruses.

State actors (and the sub-state groups they sponsor) are currently the only groups with the resources, expertise, motivation, and time to leverage this dual-use information. These states could protect their own populace from a global pandemic by secretly stockpiling vaccines that are protective against their modified strain. For this reason, states would be more likely to produce modified influenza viruses than coronaviruses (because no vaccines exist for this type of agent) and would probably be uninterested in developing strains able to overcome any vaccine (as this strain would vitiate their comparative

advantage). Sub-national malicious actors may obtain the capability to replicate some of the less complex GoF studies, but have so far not demonstrated any capacity to work with viral agents and little capacity for waging biological warfare in general. Highly skilled individuals trained in biology would be capable of replicating GoF studies, but are currently constrained greatly by a lack of material resources and time

Finally, no information risks unique to GoF research were identified. Similar techniques to those used in GoF experiments could be leveraged for other pathogens to create a highly transmissible strain of an already deadly virus (like the Hendra and Nipah viruses) or to create a deadly strain of an already highly transmissible pathogen that has been modified to overcome protective vaccination (polio-, mumps-, or measles-virus). Perhaps most worryingly, reverse genetics techniques could be used to synthesize smallpox virus if an actor has significant molecular biology skill, and this strain could be modified to overcome protective vaccination. Non-GoF pathogens could be used to produce effective, novel incapacitating agents by the modification of a highly contagious virus (polio-, mumps- or measles-virus) to overcome protective vaccination.

8.2 Purpose and Approach

The purpose of this task is to identify those GoF studies on influenza, SARS, and MERS viruses that, if published, would provide useful information to a malicious actor seeking to create a biological weapon. This analysis assumes that the body of dual-use information already in the public domain is significant and so seeks to identify studies that would contribute to the ability of a malicious actor beyond what has already been published. Since an adversary is presumably interested in causing harm in any way possible, this analysis considers GoF studies on influenza, SARS-CoV, and MERS-CoV in light of what can already be achieved with unmodified strains of these pathogens and non-GoF pathogens. Indeed, the capability to cause harm with agents other than influenza virus, SARS-CoV, and MERS-CoV is significant. Hence, this comparative assessment must be conducted to understand the advantage an adversary gains by leveraging the information gleaned from GoF studies, specifically. Lastly, to provide insight into the possibility that novel, dual-use information would be exploited if it were published, this study examines the capability and motivation of malicious actors to weaponize pathogens.

8.3 Methods

8.3.1 Use of Sources

This biosecurity information risk assessment involves the analysis of the biosecurity risk posed by the future publication of GoF research results beyond the existing dual-use information already in the public domain. This analysis uses scientific data to identify potential new capabilities afforded by GoF research to those who seek to cause harm. Biomedical literature describes the infectiousness, pathogenicity, and countermeasure resistance of wild type pathogens, and potential modifications to pathogens to enhance any of these traits. Information from intelligence/law enforcement data was used to provide the general context necessary to understand the capabilities of malicious actors to exploit this research but could not be directly reported at an unclassified level. Beyond this contextual level of discussion, we relied on open-source information on offensive biological weapons programs undertaken by states and non-state actors to source our analysis of malicious actor intent and capability.

8.3.2 Methodology for Baselining the Biological Threat

We first conducted an analysis of the biomedical literature and open-source descriptions of state-sponsored offensive weapons programs to determine what a malicious actor using unmodified agents

could achieve. We then examined how GoF pathogens could provide *additional* capabilities to an adversary. In this analysis, we considered the ability of various pathogens to incapacitate and kill as the possible desired outcomes of a biological attack. Attacks targeting animals for the purpose of causing economic harm or harm to animal health were outside the scope of the assessment. We also considered the "footprint" of the attack, meaning the area and time over which the attack would incapacitate or kill (under the assumption that a larger area or time of effect was desirable). Contagiousness of GoF pathogens is considered in this context. Given this baseline, our analysis identifies the type of information created via GoF studies that would prove useful to adversaries seeking to build additional biological weapon capabilities.

One quantitative method was used to baseline the threat. To quantitatively assess the dual-utility of the phenotype of enhanced growth, we compared how the number of victims infected from an intentional release scaled with the total amount of pathogen aerosolized, which itself is a function of how much pathogen can be produced. To perform this assessment, we used the Hazard Prediction and Analysis Capability (4.0) as described in the Risk Assessment of Accidents and Natural Disasters section above. We modeled only New York City as the target (due to its population density) across 12 different weather conditions for each release amount to show the maximum extent of a large attack.

8.3.3 Methodology for Baselining the State of the Science

Given that very little can be done about the dual utility of studies already published, we characterized the state of the science regarding the enhancement of all traits described in the NSABB framework. We analyzed the body of literature that encompasses all GoF studies identified by the project team for the benefit assessment and/or risk assessment (see bibliography). Specifically, we sought to understand to what degree the methods for the creation of modified strains of influenza viruses and coronaviruses with the following phenotypes already exist in the public domain:

- Enhanced production of pathogens *in vitro* or *in ovo* (high titer),
- Enhanced mortality,
- Enhanced morbidity,
- Enhanced transmission in mammals,
- Evasion of natural or induced immunity, and
- Evasion of medical countermeasures, including vaccines, antivirals and diagnostics.

This task culminated with the identification of GoF research that would provide uniquely valuable information to a malicious actor for misuse beyond the body of dual-use research that already exists. Also, we identified whether dual-use information already in the literature requires a particularly challenging technical approach in order to ascertain if a biosecurity information risk could be suffered via the publication of an easier experimental route to the same product. Similarly, instances in which the researchers published the specific genetic changes leading to the desired traits are noted because a malicious actor could simply recreate the useful strains using reverse genetics instead of repeating the methods. This section highlights which of the phenotypes described under the funding pause have yet to be achieved in the published literature, representing a remaining, possible information risk.

8.3.4 Evaluation of the Capability and Intent of Malicious Actors to Leverage Dual Use Information

We used open-source information to characterize the technical skill, sophistication, and resources required to replicate those GoF experiments that provide information uniquely useful and of interest to a malicious actor. We relied on historical precedent, as documented in open source information, in

considering whether certain malicious actors might have intent to leverage uniquely dual use information yet to be published.

8.4 Baselining the Biological Threat

When considering the information produced by GoF experiments, we considered how the results achieved intersect with the goals of those wishing to misuse the information. As in other sections of this report, we used the NSABB definition of GoF research for this analysis.[397] Specifically, we consider various strains of seasonal, pandemic, and avian influenza and the MERS and SARS coronaviruses. The phenotypes we consider are:

- Enhanced production of pathogens *in vitro* or *in ovo* (high titer),
- Enhanced mortality,
- Enhanced morbidity,
- Enhanced transmission in mammals,
- Evasion of natural or induced immunity, and
- Evasion of medical countermeasures, including vaccines, antivirals and diagnostics.

From the perspective of an adversary seeking to create a biological weapon (called a "weaponeer") these phenotypes can be described by three agent/weapon characteristics. **Mortality** covers the GoF phenotype of enhanced mortality and the ability of a pathogen to evade medical countermeasures and natural or induced immunity, as its ability to do so increases the overall case fatality rate. **Incapacitation** covers the GoF phenotype of enhanced morbidity, but also the phenotypes describing the evasion of medical countermeasures and natural or induced immunity, as these abilities increase the attack rate or the severity or duration of illness. **Footprint**—the ability of a weapon to cover an area, extend a pathogen's effects over time, or to reach a set number of victims—encompasses several GoF phenotypes. A strain with enhanced production characteristics can be used to increase the effective payload of a weapon (i.e., the same production run can produce more pathogen), potentially infecting more victims and covering a larger area when the agent is released using a weapon. A highly contagious GoF strain increases the footprint of an attack by increasing the number of victims harmed after the primary aerosol, which, in turn, increases the geographic and temporal extent of the effects. Similarly, a strain that evades medical countermeasures increases the number of victims potentially harmed by the primary aerosol. For contagious strains, the evasion of medical countermeasures also increases the attack rate and geographic and temporal extent of a resulting outbreak compared to an outbreak that can be effectively controlled by medical countermeasures.

To understand how GoF research could provide information that increases the ability of a weaponeer to produce a weapon that is highly lethal, highly incapacitating, or has a large footprint, we compare these GoF outcomes with what is possible without GoF research. We first consider the phenotypes separately and then consider under which circumstances the combination of traits leads to a particularly useful strain.

[397] Framework for Conducting Risk and Benefit Assessment of Gain-of-Function Research: Recommendations of the National Advisory Board for Biosecurity. May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

8.4.1 Mortality from Diseases Caused by Non-GoF Pathogens

Without any information from GoF research, a weaponeer can choose from several agents that cause diseases with extremely high mortality rates, which can be identified simply by scrutinizing the Select Agent list.[398]

8.4.1.1 The Bacterial Agents

Several diseases caused by bacterial agents have an extremely high case fatality rate. Inhalational anthrax has an untreated case fatality rate of 90% and a treated case fatality rate of approximately 50% if aggressive treatment is provided.[399] Melioidosis has a case fatality rate in western countries of about 15%, although many of the victims have significant co-morbidities.[400,401] Pneumonic plague is almost uniformly fatal if untreated.[402] Although the untreated case fatality rate of the typhoidal form of tularemia is about 30%, animal studies suggest that high doses that may be experienced in the context of a biological attack significantly increase the lethality of this agent.[403,404]

Because all of these agents are bacteria that can replicate outside of a host cell, a weaponeer would likely find isolating, growing, and weaponizing these agents easier than the influenza viruses and coronaviruses.[405] Also, since many of the bacterial Select Agents featured in the offensive weapons programs of several states, information on their efficient weaponization already could be available or obtained by state actors.[406,407,408]

From a weaponeer's perspective, the disadvantage of using bacterial agents is that the diseases they cause can be prevented or effectively treated with antibiotics. However, simple molecular or microbiological methods (such as selection *in vitro* or *in vivo*) can be used to induce significant resistance in these bacteria to a panel of therapeutically useful antibiotics. Moreover, the methods to eliminate the fitness defect associated with newly acquired antibiotic resistance (or indeed any newly acquired phenotype) also involve relatively simple microbiological manipulations. In short, when compared to methods related to

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398 US Government Publishing Office, "Title 42: Public Health. §73.3 HHS Select agents and toxins," http://www.ecfr.gov/cgi-bin/text-idx?SID=a2b0afcad59ea49b88e4bf9e9b20a26c&mc=true&node=pt42.1.73&rgn=div5#se42.1.73_13.

399 Jon-Erik C. Holty et al., "Systematic review: a century of inhalational anthrax cases from 1900 to 2005," *Annals of Internal Medicine* 144 no. 4 (February 2006): p. 270-280, http://annals.org/article.aspx?articleid=720551.

400 Saidani N, et al., "Melioidosis as a travel-associated infection: Case report and review of the literature," *Travel Medicine and Infectious Disease* (4 September 2015) http://www.sciencedirect.com/science/article/pii/S1477893915001428.

401 Nasser-Posso K, et al., "Human melioidosis reported by ProMED," *International Journal of Infectious Diseases* 35 (June 2015): p.103-104, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4508390/

402 Kiersten J. Kugeler et al., "Epidemiology of Human Plague in the United States, 1900-2012," *Emerging Infectious Diseases* 21, no. 1 (January 2015): p. 16-22, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4285253/.

403 Joseph R. Egan, Ian M. Hall, Steve Leach, "Modeling Inhalational Tularemia: Deliberate Release and Public Health Response," *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science* 9, no. 4 (2011): p.334-335, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3223019/pdf/bsp.2011.0004.pdf.

404 Glynn A, et al., (2015) "Comparison of experimental respiratory tularemia in three nonhuman primate species," Comparative Immunology, *Microbiology and Infectious Diseases* 39 p. 13-24, http://www.ncbi.nlm.nih.gov/pubmed/25766142.

405 This argument was made in general form in: Jonathan B. Tucker, "Bioterrorism: Threats and Responses," *Biological Weapons: Limiting the Threat*, ed. Joshua Lederberg (Cambridge: The MIT Press, 2001), p. 286.

406 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.

407 United Nations Monitoring, Verification and Inspection Commission (UNMOVIC), "Compendium: Chapter V, the Biological Weapons Programme," retrieved at: https://web.archive.org/web/20131203182832/http://www.un.org/depts/unmovic/new/documents/compendium/Chapter_V.pdf.

408 The Secretary of Defense, "Memorandum For the President, National Security Decision Memoranda 35 and 44," July 6, 1970, Declassified http://usarchive.gwu.edu/NSAEBB/NSAEBB58/RNCBW22.pdf.

GoF, a weaponeer can much more easily obtain a highly lethal strain of bacteria via simple methods of manipulation.

8.4.1.2 The Viral Agents

Several viral agents on the Select Agent list are associated with a high case fatality rate. The Hendra and Nipah viruses have a case fatality rate of roughly 50%, with no effective treatment.[409,410,411,412] Marburg virus, a hemorrhagic fever virus (HFV) on the Select Agent list that was weaponized by the Soviet Union, had a 22% case fatality rate in Europe during its initial outbreak and rates above 80% in subsequent outbreaks in the developing world.[413,414] Hemorrhagic fever case fatality rates are worsened by the difficulty of applying proper clinical care management and the lack of non-experimental treatments with demonstrated efficacy.[415] Since Marburg HFV featured in the offensive biological weapons program of the Soviet Union, a malicious state-level actor may be able to access the methods to magnify and weaponize this agent.[416] Although not a select agent, rabies virus causes a nearly uniformly lethal infection if not prevented by vaccination soon after exposure. Finally, while currently circulating strains of H5N1 avian influenza are associated with a fatality rate of 60%, this rate may be inflated due to potentially unreported cases of mild illness in this outbreak.[417,418] SARS and MERS outbreaks are associated with a case fatality rate greater than 10% as well, albeit mostly in the elderly (see Section 4).[419] In short, a weaponeer can use a variety of wild type viruses if high mortality is desired without resorting to the exploitation of more sophisticated GoF methods.

> Several bacterial and viral agents give the weaponeer a choice of pathogens that are highly lethal without relying on information from GoF experiments.

8.4.1.3 Toxins

Several toxins are listed on the Select Agent list.[420] These toxins are highly deadly and lack effective treatments for victims who have received a sufficiently large dose. Extracting or otherwise producing enough toxin from biological organisms to inflict a mass casualty requires industrial-like production capacity. That being said, several state actors and one sub-state actor have invested in the capacity to

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409 Broder C, et al., "A treatment for and vaccine against the deadly Hendra and Nipah viruses."
410 Centers for Disease Control (CDC), "Hendra Virus Disease (HeV): Treatment." http://www.cdc.gov/vhf/hendra/treatment/index.html
411 Playford E, et al., "Human Hendra Virus Encephalitis Associated with Equine Outbreak, Australia, 2008," Emerging Infectious Diseases 16, no. 2 (February 2010), http://wwwnc.cdc.gov/eid/article/16/2/09-0552_article.
412 Robin McConclue, "Hendra trials for humans about treatment not prevention," *ABC Rural*, April 1, 2015, http://www.abc.net.au/news/2015-04-01/human-hendra-drug-treatment-not-prevention/6365472?WT.ac=localnews_brisbane.
413 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.
414 Mehedi M, et al., (2011) "Clinical aspects of Marburg hemorrhagic fever," *Future Virology* p. 1091-1106, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3201746/.
415 Ippolito G, et al. (2012) "Viral hemorrhagic fevers: advancing the level of treatment." *BMC Medicine* 10, no. 31 www.biomedcentral.com/1741-7015/10/31.
416 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.
417 Bui C, et al, (2015) "A Systematic Review of the Comparative Epidemiology of Avian and Human Influenza A H5N1 and H7N9- Lessons and Unanswered Questions," *Transboundary and Emerging Diseases* p.6, http://onlinelibrary.wiley.com/doi/10.1111/tbed.12327/references.
418 Morens D, Taubenberger J (2015) "How Low Is the Risk of Influenza A (H5N1) Infection?," *The Journal of Infectious Diseases* 211, no. 9 p. 1364-1366, http://jid.oxfordjournals.org/content/211/9/1364.long.
419 Guan Y *et al* Molecular epidemiology of the novel coronavirus that causes severe acute respiratory syndrome. *The Lancet* 363: 99-104
420 US Government Publishing Office, "Title 42: Public Health. §73.3 HHS Select agents and toxins."

produce toxins in quantities useable in weapons.[421,422] Therefore, for adversaries willing to invest in industrial scale production of toxins, GoF information provides little value for achieving a highly lethal agent because toxins are already very deadly.

8.4.2 Incapacitation from Diseases Caused by Non-GoF Pathogens

Some biological weapons are not designed to kill, but rather to incapacitate the soldiers or industrial workers of an enemy. In fact, incapacitating agents were featured heavily in the now defunct US offensive biological weapons program.[423] Due to their high case fatality rate, MERS and SARS coronaviruses cannot be considered incapacitating agents and are not discussed further in this section. The effectiveness of an incapacitating agent can be described by three characteristics: the infectious dose, the severity of the symptoms, and the duration of incapacitation.

Wild type strains of the incapacitating agents weaponized in the former US offensive biological weapons program, such as *Coxiella burnetii*, have a number of characteristics that make them suited for this purpose. The median infectious dose in humans of *C. burnetii* is less than ten microbes, which is comparable to the most infectious strains of influenza and the coronaviruses and provides little opportunity for improvement utilizing information from GoF studies.[424] The symptoms of the acute disease caused by infection with *C. brunetii*, called Q fever, are similar in severity and type to influenza, including a high fever (up to 105°F), pain, headache, malaise, vomiting, and diarrhea.[425] These symptoms persist longer than the symptoms of influenza, with fever typically lasting longer than ten days (fever in influenza lasts typically half as long—see Supplemental Information on the disease course of influenza).[426] Moreover, some victims develop a chronic form of Q fever with long-lasting and recurrent disabling symptoms.[427] Antibiotics can be used to effectively treat the illness, but, as described above, antibiotic resistance can be imbued into this agent using methods much less technically challenging than those necessary to undertake GoF studies. Moreover, because *C. burnetii* was weaponized in the former US weapons program, the information needed to grow and weaponize this agent could be leveraged by an adversary.[428,429] In short, GoF studies with the influenza viruses are unlikely to lead to the development of a pathogen that is more effective as an incapacitating agent than *C. burnetii* because this agent is highly infectious and produces a severe, relatively long-lasting illness. The only caveat is that influenza infections have a relatively fast symptom onset time compared to *C. burnetii* infections (an average of two days for influenza infections, versus two to three

> GoF studies with the influenza viruses are unlikely to lead to the development of a pathogen that is better as an incapacitating agent than *C. burnetii*, unless rapid symptom onset times are desired by a malicious actor above all other characteristics.

421 United Nations Monitoring, Verification and Inspection Commission (UNMOVIC), "Compendium: Chapter V, the Biological Weapons Programme,"
Michael A. Guhin to Robert M. Behr, "Memorandum for Dr. Kissinger, Subject: The Toxins Issue."

422 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press

423 See Tab A: Material to be Destroyed (Biological and Toxin), in
The Secretary of Defense, "Memorandum For the President, National Security Decision Memoranda 35 and 44," July 6, 1970, Declassified, p.3, http://nsarchive.gwu.edu/NSAEBB/NSAEBB58/RNCBW22.pdf.

424 Russell John Brooke et al., "Human dose response relation for airborne exposure to Coxiella burnetii," *BMC Infectious Diseases* 13, no. (2013): http://www.biomedcentral.com/1471-2334/13/488.

425 Centers for Disease Control and Prevention, "Q Fever: Symptoms, Diagnosis, and Treatment," November 13, 2013, http://www.cdc.gov/qfever/symptoms/.

426 Ibid.

427 Ibid.

428 The Secretary of Defense, "Memorandum For the President, National Security Decision Memoranda 35 and 44," p.3.

429 William J. Broad, "US Selling Papers Showing How to Make Germ Weapons," *The New York Times*, January 13, 2002, http://www.nytimes.com/2002/01/13/national/13GERM.html.

weeks for acute symptom onset for *C. burnetii* infections), and a malicious actor that strongly values rapid effects over other weapons characteristics may favor influenza. In this case, wild type influenza viruses may be considered nearly ideal.[430]

8.4.3 Footprint of Attacks of Non-GoF Pathogens

As described above, the footprint of an attack is defined as the number of victims affected, the physical area affected, or the duration of the disruption directly caused by the attack. In Section 8.4.3.1 below, we explore how GoF phenotypes could affect the footprint of a weapon. In this section, we explore how wild type pathogens and existing technology can create large footprint attacks.

The biological agent that most notoriously embodies attributes desirable for large footprint attacks is *B. anthracis*, which led to its inclusion in several state offensive weapons programs.[431,432,433] The spores formed by *B. anthracis* are extremely resistant to environmental forces and can survive for a long time suspended in an aerosol.[434] This pathogen is able to create large footprint attacks, which is demonstrated by the use of the related organism, *B. thuringiensis*, in pest control programs involving the treatment of square miles of territory with spores dispensed from a single vehicle.[435] If dispersed by sophisticated maritime, ground-based, or aerial platforms, *B. anthracis* could cover thousands of square miles and reach millions of people with a single attack (as demonstrated by a series of pre-1969 US tests using simulants, such as Operation Large Area Coverage).[436,437] Although the biological properties of non-contagious agents can *facilitate* their use in a weapon that can attack large areas, the ability of a non-contagious agent to reach these large areas is highly dependent on the dispersal system, which require sophisticated engineering skills to develop.[438] Conversely, contagious agents could expose (and possibly infect) large numbers of people over a wide area through the ongoing outbreak and the movement of infected people without the need for a sophisticated dispersal device.

Insofar as an attack is desired to cause disruption for a long period of time, *B. anthracis* is also a good candidate because its spores can persist in buildings or in the soil for years. For instance, two and a half months were required to perform decontamination operations at the Landover

> Existing non-contagious agents are very good at causing mass casualties or contaminating a large area for a long period of time if dispersed from a very sophisticated device. Contagious agents could have similar consequences with very simplistic dispersal methods.

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430 Centers for Disease Control and Prevention, "Clinical Signs and Symptoms of Influenza: Influenza Prevention & Control Recommendations." <http://www.cdc.gov/flu/professionals/acip/clinical.htm>;
Centers for Disease Control and Prevention, "Q Fever: Symptoms, Diagnosis, and Treatment."

431 United Nations Monitoring, Verification and Inspection Commission (UNMOVIC), "Compendium: Chapter V, the Biological Weapons Programme".

432 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press

433 The Secretary of Defense, "Memorandum For the President, National Security Decision Memoranda 35 and 44,"

434 Jonathan B. Tucker, "Bioterrorism: Threats and Responses," p. 286.

435 Sheila Van Cuyk et al., "Persistence of *Bacillus thuringiensis* subsp. *kurstaki* in Urban Environments following Spraying," Applied and Environmental Microbiology 77 (2011): p. 7954-7961, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3208992/.

436 Cole L, (1998) *Clouds of Secrecy: The Army's Germ Warfare Tests Over Populated Areas* Lanham: Rowman & Littlefield.

437 van Courtland Moon J, (2006) "The US Biological Weapons Program." *Deadly Cultures: Biological Weapons since 1945*, Cambridge: Harvard University Press.

438 For instance, the terrorist group Aum Shinrikyo tried to manufacture their own vehicular spray system, with poor result. Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," *Center for a New American Security*, December 2012, p. 27, http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf.

mail facility in the aftermath of the 2001 anthrax attacks.[439] Gruinard Island, the site of testing of anthrax weapons in the former UK offensive weapons program, was dangerously contaminated for decades after testing ceased.[440] The entire topsoil of the island had to be decontaminated before the site was considered safe for human occupation.[441] Although the biological properties of *B. anthracis* enable a weaponeer to deny access to a particular location for a long time after an attack, a population may simply avoid the contaminated area and remain safe. In contrast, a contagious agent prolongs the effect of an attack because the population itself carries the hazard, and, therefore, risk can be minimized only by reducing human contact.

8.4.3.1 Contagiousness as a Desirable Characteristic to Increase the Footprint of an Attack

From the analysis above, the acquisition of a contagious agent would enable a weaponeer to expand the footprint of their attack in terms of casualties, area, and time. Moreover, the fact that an outbreak can spread, sickening or killing victims beyond those infected by the initial attack, removes the requirement to produce a sophisticated dispersal device to cause a mass casualty attack. For this reason, contagious agents may be desirable by malicious actors who have significant skill in virology but no skill in machining/engineering to make a munition.

However, contagious agents have drawbacks—primarily that their affect is difficult to predict or control. Some state and sub-state groups may not desire an agent that could infect their soldiers or supportive populations, or could cause mass fatalities. For instance, the United States' now defunct offensive biological weapons program considered a potential agent's ability for human-to-human transmissibility as a negative characteristic.[442,443] This feature may be especially problematic if their supporters live primarily in low-income countries because global outbreaks there may be more severe than in high-income countries where the public health infrastructure is more robust. However, unlike the United States, the Soviet Union's program sought out highly contagious pathogens for at least some of the lethal pathogens in its arsenal.[444] At the state actor level, weaponeers may covertly stockpile a vaccine to mitigate friendly losses to their contagious agent.

If a contagious, lethal agent is desired by a weaponeer, they could choose to work with a wild type agent other than the influenza viruses or coronaviruses. Of the non-GoF Select Agents, smallpox virus, *Yersinia pestis*, and the filoviruses (Ebola and Marburg viruses) are the only viruses that have a high case fatality rate and are significantly contagious. *Y. pestis* causes pneumonic plague if inhaled by a victim. This pathogen is often described as being highly transmissible, with a historical R_0 at or above that for influenza strains.[445,446,447] However, these historical studies draw upon past outbreaks in areas that do not

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439 Dorothy A. Canter et al., "Remediation of Bacillus anthracis Contamination in the US Department of Justice Mail Facility," *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science* 3, no. 2 (June 2005): p. 119-127, http://online.liebertpub.com/doi/abs/10.1089/bsp.2005.3.119.

440 "Britain's 'Anthrax Island'," *BBC News*, July 25, 2001, http://news.bbc.co.uk/2/hi/uk_news/scotland/1457035.stm.

441 Ibid.

442 US Department of the Army, "US Army Activity in the US Biological Warfare Programs, Volume 1," February 24, 1977, Unclassified, p. 50-51, http://nsarchive.gwu.edu/NSAEBB/NSAEBB58/RNCBW_USABWP.pdf.

443 Leitenberg M, Zilinskas R. (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press

444 Ibid

445 Gani R, Leach S, (2004) "Epidemiologic determinants for modeling pneumonic plague outbreaks," *Emerging Infections Diseases* 10, no. 4 p.608-614, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3323083/

446 Nishiura H, et al., (2006) "Transmission potential of primary pneumonic plague: time inhomogeneous evaluation based on historical documents of the transmission network," *Journal of Epidemiology and Community Health* 60, no.7 p.640-645, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2566243/

447 Coburn BJ, Wagner BG, Blower S, (2009) "Modeling influenza epidemics and pandemics: insights into the future of swine flu (H1N1)," BMC Medicine http://www.biomedcentral.com/content/pdf/1741-7015-7-30.pdf

mirror the modern US.[448,449] Indeed, most of the secondary cases of pneumonic plague described occurred in household members of the ill, who also were the primary caregivers at a time when hospitalization of the critically ill was still rare. Estimates of the R_0 of pneumonic plague in the modern United States suggest that an outbreak would extinguish rather rapidly.[450] Similarly, outbreaks of Ebola virus disease in Africa were associated with an R_0 that approached that of influenza or SARS.[451,452,453] However, much of the transmission was in makeshift healthcare facilities suggesting that the R_0 in the US would be much smaller.[454,455] Smallpox virus is held in just a few, highly secure locations throughout the world and, therefore, may be difficult for an adversary to acquire. That being said, an adversary able to leverage the information produced by GoF experiments may well be able to use non-GoF approaches to synthesize smallpox virus *de novo* using well described rescue systems for other orthopoxviruses.[456] Similarly, although there is no environmental reservoir of SARS-CoV, the significant transmissibility and lethality of the wild type strain may motivate a weaponeer to use reverse genetics to synthesize it or attempt to acquire it from a laboratory.

If a contagious, incapacitating agent is desirable, not many options to acquire such an agent are available. Wild type pandemic or seasonal influenza viruses are obviously highly contagious and incapacitating, however, residual immunity from past outbreaks may hamper the spread of these illnesses.[457,458] An adversary could choose a wild type strain that has not circulated in several decades to reduce the effect of residual immunity in the population. Alternatively, modified strains of many pathogens, including mumps virus and measles virus, which are already highly contagious, could be made to overcome protective immunity using techniques similar to those used in GoF experiments. However, these experiments would be associated with their own information risk and, therefore, are not explored further here.

> A contagious pathogen may be desirable by a scientifically trained adversary with minimal engineering skill or by a state. Smallpox virus and SARS-CoV are the only wild type pathogen that is deadly and as transmissible (or more) than influenza virus. Wild type influenza viruses are contagious, especially if a decades old strain is used to minimize the effect of residual immunity, and highly incapacitating.

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448 Gani R, Leach S, (2004) "Epidemiologic determinants for modeling pneumonic plague outbreaks," *Emerging Infectious Diseases* 10, no. 4 p.608-614, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3323083/.

449 Hinckley AF, et al., (2012) "Transmission dynamics of primary pneumonic plague in the USA," *Epidemiology and Infection* 140, no. 3 p. 554-560.

450 Ibid.

451 Adnan Khan et al., "Estimating the basic reproductive ratio for the Ebola outbreak in Liberia and Sierra Leone," *Infectious Diseases of Poverty* 4, no. 13 (February 2015), http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347917/.

452 G. Chowell et al., "The basic reproductive number of Ebola and the effects of public health measures: the cases of Congo and Uganda," *Journal of Theoretical Biology* 229, no. 1 (July 2004): p.119-126.

453 Zhi-Qiang Xia et al., "Modeling the transmission dynamics of Ebola virus disease in Liberia," *Scientific Reports* 5 no. 13857 (September 2015): p. 1-13, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4561958/pdf/srep13857.pdf.

454 Ibid.

455 see also: Joseph A. Lewnard et al., (2014) "Dynamics and control of Ebola virus transmission in Montserrado, Liberia: a mathematical modelling analysis," Lancet Infectious Diseases 14, no. 12 p. 1189-1195, http://www.thelancet.com/pdfs/journals/laninf/PIIS1473-3099%2814%2970995-8.pdf

456 See FOUO addendum for examples.

457 For evidence of residual immunity, see for example: Pérez-Trallero E. (2009) "Residual Immunity in Older People Against the Influenza A(H1N1) - Recent Experience in Northern Spain," *Eurosurveillance* 14, no. 39 http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19344.

458 On the effect of residual immunity on spread, see for example: Xu-Sheng Zhang et al., (2015) "Co-circulation of influenza A virus strains and emergence of pandemic via reassortment: the role of cross-immunity," *Epidemics* 5, no. 1 p. 20-33.

8.4.3.2 Enhanced Growth as a Desirable Characteristic to Increase the Footprint of an Attack

Because pathogens are self-replicating, to increase the footprint of an attack, an adversary could simply grow more of the agent. This goal *could* be accomplished by increasing the volume of culture used or by increasing the viral titer in the culture. This rationale supports the consideration of enhanced growth of a pathogen in culture or eggs as a dual-use phenotype because an adversary can produce more pathogen with the same amount of resources if the pathogen can grow to a high titer. We therefore evaluated how producing more pathogen for a biological attack, in terms of the amount of pathogen released, relates to the footprint of the attack, particularly in terms of number of victims infected (data is provided in the FOUO addendum). The results show that, for small amounts of pathogen effectively released, the number of initial infections scales well with the amount of pathogen produced (that is, tenfold more pathogen released leads to tenfold more initial infections). For larger amounts of pathogen, the increase in casualties begins to taper off as the amount of pathogen released increases. Specifically, for every tenfold increase in pathogen released, the number of initial infections produced increases by only two- to three-fold or less. Our results show that increasing the growth of a wild type strain will have a limited effect on the amount of casualties produced even for actors who do not have access to industrial scale production facilities. In short, even relatively poorly growing pathogenic strains of influenza virus grow well enough such that producing more pathogen produces limiting returns for use in an attack.

Moreover, if a malicious actor wants to infect as many people as possible, spending the effort to modify a strain to become high growth is probably not worth the risk that its transmissibility would be decreased. By their nature, transmissible agents would increase the footprint of the attack, potentially by several orders of magnitude if an ongoing and global outbreak can be sparked. An attack that initially infects several thousand people will quickly grow out of local control (as demonstrated in the Risk Assessment of Accidents and Natural Disasters), and therefore, the number of casualties produced by the attack will depend on the pathogenicity and transmissibility of the pathogen in the context of a global epidemic and not the initial number infected. In Chapter 5, it is demonstrated that very few covert infections of the public are required to seed nearly guarantee that an outbreak would escape local control for the influenza viruses (ten or fewer), so the initial number of people exposed could be very small indeed.

> Experiments that enhance the growth in culture of the GoF pathogens are of minimal information risk because producing more agent results in few additional casualties.

8.4.3.3 Countermeasure Resistance and Evasion of Existing Immunity as Desirable Characteristics to Increase the Footprint of an Attack

The GoF phenotype of evasion to medical countermeasures includes the ability to evade the protection afforded by vaccines, antivirals, and diagnostics. The evasion of diagnostics is not particularly relevant to GoF diseases released intentionally. SARS and MERS diagnostics are used in the US only for people already thought to be infected with the disease based on their clinical symptoms.[459] Since no specific treatments for these diseases currently exists, these diagnostics would simply be used to direct public

[459] Centers for Disease Control and Prevention, (2014) "Middle East Respiratory Syndrome (CDC) - CDC Laboratory Testing for Middle East Respiratory Syndrome Coronavirus (MERS-CoV)," http://www.cdc.gov/coronavirus/mers/lab/lab-testing.html.

health measures.[460,461,462] Given the importance of quarantine and isolation in the control of coronavirus outbreaks, diagnostics could be used to direct public health resources to prevent the outbreak escaping local control.[463]

Because pandemic and seasonal influenza viruses are highly contagious, the evasion of diagnostics would have little consequence to the eventual extent of an outbreak, but may complicate the efficient use of antivirals by confounding the identification of patients who are infected with influenza. For strains of avian influenza modified to be contagious between humans, the evasion of a diagnostic would have just as few benefits to a malicious actor as it would for the use of a coronavirus.

In an intentional attack with influenza virus, evasion of immunity induced by vaccination is of little relevance because we presume the attack would be a surprise and the US would not have prepared sufficient stocks of a protective vaccine ahead of time. For this reason, several months would pass before the vaccine would be ready for deployment, at which time the disease would have spread globally (or, if poorly contagious, extinguished by itself). However, the vaccine could still be used to limit the casualties and temporal duration of the outbreak (see Figure 9.5 in Chapter 9 to see how the timing of the deployment of a vaccine affects global casualties). However, a virus that can overcome protective immunity induced by *any* vaccine would increase both the casualties of an attack and its duration. Because no approved vaccines for the coronaviruses currently exist, malicious actors have no need to make a strain of these viruses able overcome protective vaccination.

An actor wishing to leverage a recently circulating strain of influenza may desire to modify their strain to avoid protective immunity. Residual immunity in the population can significantly reduce the chance that an outbreak would escape local control and the consequences of an outbreak (Figures 6.35 and 6.57 in Chapter 6). However, avoidance of residual immunity can be obtained either through GoF methods or by the selection of a wild type strain that has not circulated recently. Infections by the SARS and MERS coronaviruses are sufficiently rare that an adversary has no need to create a strain that can evade residual immunity from a past infection.

Antiviral resistance of influenza viruses would be useful to an adversary to increase the casualties caused by an outbreak in the United States and to increase the chance that a local outbreak escapes local control (as shown in Figures 6.53 for seasonal influenza and 6.55 for pandemic influenza in Chapter 6). Given that the majority of the world does not have access to the amount of antivirals that the United States does, antiviral resistance has little influence on global consequences.

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460 During the 2003 SARS-CoV epidemic, Ribavirin was used; however, it "did not appear to have a significant effect," and a study of patients treated with Ribavirin indicated "that ribavirin provided no benefit in the resolution of symptoms or survival." In: Els Keyaerts, Leen Vijgen, Marc Van Ranst, "Current Status of Antiviral Severe Acute Respiratory Syndrome Coronavirus Research," *Coronaviruses: Molecular and Cellular Biology*, ed. Volker Thiel (Norfolk: Caister Academic Press, 2007), p. 328.

461 Centers for Disease Control and Prevention, (2015) "Middle East Respiratory Syndrome (MERS)" http://www.cdc.gov/coronavirus/mers/about/prevention.html.

462 World Health Organization, (2013) "Severe Acute Respiratory Syndrome (SARS)". http://www.who.int/immunization/topics/sars/en/

463 See in particular figure 2 in: Simon Cauchemez et al., "Middle East respiratory syndrome coronavirus: quantification of the extent of the epidemic, surveillance biases, and transmissibility," *Lancet Infectious Diseases* 14, no. 1 (January 2014): p. 50-56.

8.4.4 Gain of Function Strains Compared to Naturally Occurring Strains

8.4.4.1 A Non-Unique Information Risk Inheres in Experiments Describing the Creation of Highly Transmissible, Highly Deadly Strains of Pathogens

When compared to strains of pathogens created via the use of GoF research information, we find that naturally occurring strains generally are not both highly pathogenic and highly transmissible (with the exception of wild type SARS-CoV). Although SARS-CoV has an R value greater than one, the fact that the virus is transmissible only after symptoms present, the disease is largely spread within the medical system, and the long incubation period of the disease makes outbreaks of SARS-CoV relatively easy to control (as described in Chapter 4, past outbreaks have witnessed a two-fold drop in the R value of a SARS outbreak after control measures are implemented). Only smallpox virus, which exists only in a few laboratories, has a case fatality rate greater than 10% and is transmissible enough to cause a pandemic (an R value significantly greater than one in the context of a robust public health response). Because stocks of smallpox virus are tightly controlled, an adversary might turn to GoF studies to acquire a pathogen that is both highly pathogenic and highly transmissible. We note that no GoF study to date has conclusively produced strains with the combination of the desired phenotypes because of weaknesses of animal models in predicting pathogenesis in humans. Moreover, whether enhancement of a phenotype, like transmissibility, would be sufficient to achieve the traits desirable by a weaponeer for an attack on a human population remains unclear. That is, an experiment may show an increase in the transmissibility of a pathogen among ferrets, but this observation cannot be translated into a specific R_0 value in a human population (or if the increase is sufficient to obtain the desired weapon characteristics). With these limitations in mind, the following GoF results would be of concern:

- Seasonal/pandemic influenza virus that retains its transmissibility but is modified to have a case fatality rate greater than 10%,
- Avian influenza viruses that are modified to be as transmissible as seasonal influenza but retain their high fatality rate, and
- SARS/MERS-like CoV that is made more transmissible.

Scientific communications that detail the creation of strains with these traits would be of concern because they would provide a route to the acquisition of a pathogen as useful to a weaponeer as smallpox virus. Importantly, we have no data that speaks to the possibility that these phenotypes are achievable in the laboratory. Perhaps, due to the epidemiology of influenza and the coronaviruses, high mortality (in excess of that associated with the 1918 pandemic strain) and transmissibility are conflicting phenotypes because the very ill do not contact many others during the contagious phase of the illness outside of a hospital.[464] Moreover, these phenotypes will not emerge by chance in the laboratory. Any experiment that selects for one phenotype is likely to allow other phenotypes to drift. That is, experiments that focus on enhancing transmissibility alone are likely to arrive at viruses that are optimized for transmissibility. In those same experiments, the viruses obtained can drift to less pathogenic forms because selection for this trait is not maintained. In fact, this phenomenon was observed in the Fouchier experiment with H5N1, albeit not in the Sutton 2014 experiment with H7N1.[465,466] In contrast, experiments that do not involve selection but

[464] Interview comment by Dr. Ian Lipkin in: Donald G. McNeil Jr., "How a Mild Virus Might Turn Vicious," *The New York Times*, June 8, 2009, http://www.nytimes.com/2009/06/09/health/09flu.html?_r=0.

[465] See Table 1 in: Sander Herfst et al., "Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets," *Science* 336 no. 6088 (June 2012): p.1534-1541.

[466] "The present findings show that adaptation of the H7N1 isolate does not appear to substantially decrease the virulence of the virus." In: Troy C. Sutton et al., "Airborne Transmission of Highly Pathogenic H7N1 Influenza Virus in Ferrets," *Journal of Virology* (2014): p. 6623-6635, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054360/.

the systematic manipulation of the components of the virus, could lead to strains that have a variety of phenotypes that are enhanced compared to a parental strain. For example, influenza reassortment studies that choose one or two genomic segments from a highly transmissible influenza strain and switch them for the cognate segments from a highly pathogenic strain can result in a strain where both phenotypes are enhanced compared to the parents.[467]

Although some GoF studies could produce information of use to an adversary wishing to obtain highly transmissible, pathogenic agents, these studies are not the only means of acquiring this type of agent. Given that the sequence of smallpox virus is public, a technically sophisticated actor could use methods similar to those employed in GoF laboratories to synthesize all (or the unique parts) of the smallpox genome and use it to rescue live virus.[468] To support this effort, rescue systems are published already for the orthopoxviruses.[469]

Moreover, researchers could use pathogens not listed in the GoF framework, but manipulate the same traits considered in the framework to obtain highly transmissible and lethal strains of pathogens. Examples include:

- Strains of filoviruses or henipaviruses that retain their pathogenicity but are modified to be much more transmissible, and
- Strains of highly transmissible agents (like polio virus or measles virus—neither one of which is a select agent) that have been modified to become more deadly and/or overcome protective vaccination.

Finally, note that the reconstructed wild type strain of the 1957 influenza virus is predicted to be highly transmissible and significantly pathogenic, and methods to recreate this virus through reverse genetics are routine in influenza laboratories (as described in the Supplemental Information, 1918 influenza is less transmissible in the modern population due to the circulation of antigenically similar H1N1 strains). Similarly, the wild type strain of SARS-CoV is highly transmissible and significantly pathogenic as well (as described in Chapter 6, further enhancing the transmissibility of SARS-CoV increases the chance that a global outbreak would occur because of the susceptibility of outbreaks caused by the wild type virus to control measures). Therefore, GoF studies may be of use only if an adversary wishes to obtain a strain that is *more* pathogenic than that the 1957 pandemic influenza strain or more transmissible than SARS-CoV.

8.4.4.2 A Non-Unique Information Risk Inheres in the Experiments Describing the Creation of Highly Transmissible Strains of Influenza Virus with Specific Enhancements in Morbidity

Wild type influenza strains are well-suited for use as incapacitating agents because they have a small mean infectious dose and cause a debilitating illness with a short incubation time. However, unlike other pathogens researched in offensive weapons programs, influenza does not cause a particularly long lasting or chronic illness. For this reason, a strain of influenza that produces a much more protracted course of illness or chronic illness would provide an advantage over naturally occurring strains because no naturally occurring strain with this combination of phenotypes is known to exist.

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467 Ying Zhang et al., "H5N1 Hybrid Viruses Bearing 2009/H1N1 Virus Genes Transmit in Guinea Pigs by Respiratory Droplets," *Science* 340, no. 6139 (June 2013): p. 1459-1463, http://www.sciencemag.org/content/340/6139/1459.

468 Institute of Medicine (US), Committee on the Assessment of Future Scientific Needs for Variola Virus, "Live Variola Virus: Considerations for Continuing Research," p. 13, 132.

469 An example of a publication of a rescue system for the orthopoxviruses is given in the FOUO appendix.

As above, GoF research is not a unique pathway to obtain this type of dual-use information. For example, an adversary could produce strains of mumps, polio, or measles that can overcome protective vaccination, to obtain an agent with significant morbidity and even greater contagiousness than is currently possible with influenza virus. Moreover, the experimental means to produce viral strains that can overcome vaccination is well established. As such, this method of obtaining a highly-contagious, incapacitating agent requires relatively little "research". In contrast, the mechanisms underlying the nature of pathogenicity of influenza, and what leads to a protracted illness, are still unknown. Moreover, no research has pointed to any mechanism for chronic influenza infection and the creation of such a strain would require a long-term research effort.

8.4.4.3 Evasion of Medical Countermeasures is a Possible Future Information Risk

Evasion of medical countermeasures is a phenotype with minimal information risk today. Because no medical countermeasures for the coronaviruses are in use today, there is no need to produce a strain of these pathogens that can evade notional countermeasures. For the influenza viruses, vaccines, antivirals and diagnostics are all in use. Because an influenza virus used in an attack would only serendipitously match the seasonal vaccine produced and stockpiled by any nation, several months would pass between the time an attack occurs and the time that a vaccine is even relevant to control the outbreak. After that point, a vaccine has some utility for preventing mortality and morbidity and curtailing the outbreak (by increasing the number of contacts needed to spread the disease). But, as shown in our Biosafety Risk Assessment (Chapter 6) the evasion of vaccine-induced immunity at best provides a modest increase in total mortality.

Similarly, when considering the mortality caused in a global outbreak, at best, antivirals reduce the death rate of influenza by a few fold. However, in the US, because of our significant supplies of antivirals, deaths can be greatly reduced by their effective administration. Conversely, then, an antiviral resistant strain could increase the deaths from an attack with influenza virus in the US.

Diagnostics are currently used to direct the use of antivirals, which either are not plentiful enough globally to influence consequences significantly or are so plentiful as to not be limiting in the US. Diagnostics are also used to identify that an outbreak with a novel virus is occurring in the first place.

If an adversary wishes to use a wild type influenza strain in an attack, residual immunity from previous outbreaks may limit the footprint of the attack. However, an adversary could simply use a wild type influenza virus of a serotype that has not circulated recently to avoid this shortcoming. Once again, published sequence information, combined with well-established protocols for the rescue of influenza viruses, could be used to obtain these strains.

However, as vaccine technology advances, research on the evasion of medical countermeasures COULD become an information risk. For example, once a universal influenza vaccine is developed, the evasion of immunity induced by this vaccine may be critical for an adversary to cause an outbreak using influenza virus as a weapon. Studies in other pathogens have described the development of strains able to overcome protective vaccination due to the expression of exogenous genes (and not via escaping immune recognition). Similarly, once systems are in place to develop a vaccine against a newly identified serotype of influenza virus in a few weeks instead of a few months, evasion of induced immunity becomes more useful to an adversary. Likewise, if antivirals become more widely available globally, research on the evasion of that antiviral would pose an information risk. Clearly, as highly effective medical countermeasures for the coronaviruses are developed, studies on their evasion would pose an information risk. That being said, since these medical countermeasures do not yet exist, designing an experiment to evade them is not currently possible. None of these technologies are likely to be deployed in the next five

years. However, once information is published, it is permanently available and retains its utility far into the future. For this reason, the information risk relevant to the evasion of medical countermeasures should be continually re-evaluated.

8.4.4.4 Enhanced Growth in Culture Affords Little Information Risk

As described in Section 8.4.3.2, strains with enhanced growth *in vitro* or *in ovo* can be used to produce more agent to disperse from a weapon using the same amount of resources. However, even poorly growing pathogenic strains can be grown in enough quantity with commonplace equipment to obviate the growth of more to produce more casualties. In contrast, the use of contagious agents, by their nature, increases the footprint (in terms of area affected and illnesses) by many orders of magnitude. For this reason, publications on changes in this phenotype present very little information risk simply because the GoF pathogens are contagious.

8.4.4.5 Summary of Possible Comparative Information Risk Arising from GoF Studies

Figure 8.2, below, provides an overview of the potential information risk of GoF research.

Dual-Use GoF Phenotype	Seasonal/Pandemic Influenza	Avian Influenza	Coronaviruses
Enhanced transmissibility in mammals			
Enhanced pathogenicity in mammals			
Enhanced transmissibility while maintaining pathogenicity			
Overcoming natural or induced immunity			
Evading diagnostics			
Antiviral resistance			
Enhanced production in cell culture or eggs			

Figure 8.2. Possible information risk arising from dual-use information relevant to GoF research. White denotes that no significant information risk exists. Dark shading denotes a significant information risk, albeit a risk that is not unique to GoF research. No information risks unique to GoF research were found.

8.5 Overview of the State of the Science of Dual Use GoF Information

In this section, we discuss how the existing body of GoF research already describes methods to obtain strains of influenza- and coronaviruses with GoF traits, regardless of their true utility to a weaponeer. We synthesize these two pieces of information to arrive at our final conclusions. That is, this section describes the GoF information risk already realized through the previous publication of dual use information. To maintain this discussion at the full-and-open level we have not cited the specific papers at issue and have instead provided these in an appendix at the For Official Use Only level. Here, we simply characterize the state of the science and describe the seminal publications.

We first describe the state of the science for research on influenza and later describe the state of the science for the coronaviruses. We discuss each GoF phenotype in turn. Most scientific publications investigate morbidity and mortality simultaneously, characterizing disease outcomes such as weight loss

or fever alongside death of the animals. For this reason, we discuss morbidity and mortality jointly as the characteristic of pathogenicity.

8.5.1 State of the Science of GoF Experiments in Influenza Viruses

8.5.1.1 Enhanced Transmissibility in Mammals

Some research groups focus on understanding the factors that cause some H1N1 strains (such as the 2009 pandemic strain or 1918 pandemic strain) to be highly transmissible while other H1N1 strains (such as avian strains and the Puerto Rico 8 strain) to not transmit in mammals. These groups use a variety of methods to develop transmissible strains of H1N1 viruses, including:

- Simple reassortment, and
- Identification of mutations unique to transmissible strains followed by the use of reverse genetics to introduce these changes in a non-transmissible strain.

These experiments, while demonstrating the ability to increase the transmissibility of influenza viruses, are of limited dual utility because one of the parental strains already has the traits desired by a weaponeer (transmissibility and pathogenicity). These experiments were executed to determine what factors are sufficient to cause a poorly pathogenic virus into a highly pathogenic virus. An adversary gains no advantage using one of these modified strains compared to the parental, pathogenic strain.

In contrast, in experiments dealing with avian-origin viruses, the parental strains are either highly lethal OR highly transmissible in mammals and the manipulations described are required to obtain a strain that is lethal AND transmissible in mammals. Several papers describe methods to manipulate highly pathogenic strains of avian influenza from the H5, H7 or H9 subtype to arrive at a strain that is transmissible by droplets in mammals (typically ferrets or guinea pigs). These studies use a variety of methods including:

- Simple serial passaging in ferrets,
- Directed, sequential reassortment of an avian strain with a pandemic strain,
- Directed reassortment followed by serial passaging,
- Targeted mutagenesis followed by serial passaging, and
- Mutagenesis of HA, followed by selection based on its binding properties and the creation of a chimeric virus.

Some of these experiments require only minimal skill in virology. The serial passaging experiment could be repeated by an actor with limited skills in molecular biology to obtain a strain of avian influenza that is transmissible in mammals. For actors with significant skill in molecular biology, these transmissible, avian-origin influenza viruses could be synthesized using reverse genetics (a common practice in GoF laboratories) because all investigators have published enough information on the specific molecular changes observed in the avian-origin strains that are transmissible in mammals. Specifically, in the case where serial passaging was used, the mutations acquired by the strains of interest were published. In the case of the reassortment study, the gene segments that contributed to the transmissible strains from both parental strain were published. For this reason, to leverage the information in these studies, an actor need not repeat the experiments exactly, but simply reconstruct the viruses that the authors identified.

The abundance of literature describing the creation of avian-origin strains that are transmissible in mammals indicates that the GoF information risk for enhanced transmissibility of influenza viruses is

already realized. Further publication on this topic would likely not exacerbate this risk because existing published methods are relatively simple to replicate.

Any study, such as ours, that examines the existing literature on transmissibility of influenza viruses to determine the dual-utility of the resulting information suffers from a critical shortcoming. The published studies use too few animals in transmissibility experiments to understand exactly *how transmissible* these newly developed strains are. That is, none of these studies compared the transmissibility of these strains in a sufficient number of animals so that levels of transmissibility (compared to wild type seasonal or pandemic influenza strains) could be determined. Moreover, ferrets in isolators do not interact with each other the same way that people in a city do. For this reason, it is impossible to use laboratory experiments to conclusively determine if the increase in transmissibility observed translates to a dangerous R_0 value, which is determined retrospectively by scrutinizing human epidemiological data. This distinction is important because risk of an outbreak escaping local control and the risk of a resulting outbreak both increase significantly as an influenza virus approaches a transmissibility comparable to that of a seasonal influenza strain (see Chapter 6). Possibly all of these viruses may be somewhat transmissible, but in a human population their R_0 may be much less than one, which is the value required to cause a global epidemic.

Experiments to compare the transmissibility of wild type strains of seasonal and pandemic strains to modified strains would help determine if information risk inheres in further publications of strains that have been modified to become transmissible. If these modified strains are in fact transmissible, but much less so than seasonal or pandemic strains, then a remaining information risk would exist in further experiments that *could* identify a strain that is as transmissible as seasonal or pandemic strains.

> Several groups have published simple methods to increase the transmissibility in mammals of pathogenic strains that were previously transmissible only in avians—therefore this type of information risk is already realized

8.5.1.2 Enhanced Pathogenicity

Several papers describe virulence factors necessary for the maintenance of pathogenicity in highly pathogenic strains of influenza, such as the avian influenza and 1918 pandemic influenza viruses. Typically, these researchers identify mutations unique to the highly pathogenic strains and introduce these mutations into a less-pathogenic strain using reverse genetics. In a few experiments, researchers used pathogenic strains that were isolated from patients with severe illness, attempting to determine what characteristics made the strains infecting these patients even more pathogenic than the parental strain. Other researchers use reassortment between highly pathogenic and non-pathogenic strains to obtain a chimeric virus to identify the components of the pathogenic strain minimally necessary to confer pathogenicity into the non-pathogenic background. These experiments, while demonstrating the ability to increase the pathogenicity of influenza viruses, are of limited dual utility because one of the parental strains already has the traits desired by a weaponeer (transmissibility and pathogenicity). If an adversary has the pathogenic, transmissible strain, they gain no advantage from these manipulations.

In contrast, other experiments result in strains that are MORE pathogenic than any parental strain used. A variety of methods are used to enhance the pathogenicity of influenza viruses, including:

- Random reassortment of seasonal and pandemic H1N1 strains,
- Directed, serial reassortment of an avian-origin strain with a pandemic strain,

- Site directed mutagenesis of residues associated with increased virulence in other strains to increase the virulence of an avian strain, and
- Serial passaging in mice.

Although the serial passaging studies require minimal skill in molecular biology, they are also of marginal dual-utility due to the limitations of the mouse model system of predicting pathogenicity of a strain in humans (or ferrets).[470] That said, these experiments indicate that an adversary seeking to develop a more pathogenic virus could serially passage their strains in ferrets (or humans) to obtain a virus that is more pathogenic. However, sometimes these serial passaging experiments lead to less pathogenic strains.

For actors with significant skill in molecular biology, strains with enhanced pathogenicity could be synthesized using reverse genetics (a common practice in GoF laboratories) because all investigators have published the specific molecular changes observed in the strains with enhanced pathogenicity. For this reason, to leverage the information in these studies, an actor need not repeat the experiments exactly, but simply reconstruct the viruses that they have identified.

> Several groups have published methods that require skills in molecular biology to increase the pathogenicity of influenza virus strains—therefore this type of information risk is already realized. But a small remaining risk exists from the publication of simple methods to the same result. Another modest risk inheres in the publication of methods to modify influenza viruses to cause chronic illness.

The abundance of literature describing the creation of influenza virus strains with enhanced pathogenicity indicates that the GoF information risk for this trait is already realized. However, leveraging this information requires skill in molecular biology (specifically, reverse genetics), the appropriate facilities and equipment. The publication of simple selection methods supporting the conclusion that virulence could be increased in relevant animal models via this simple method poses a remaining information risk. In contrast, we found no publications describing the creation of an influenza virus strain that can produce prolonged or chronic illness, which is important in the context of malicious actors seeking to produce an incapacitating agent.

8.5.1.3 Enhanced Transmissibility While Maintaining/Enhancing Pathogenicity

Experiments in this category are of particular concern because they could enable a hostile actor to obtain a strain that has a combination of pathogenicity and transmissibility that surpasses all wild type, human pathogens except for smallpox virus. Several papers describe methods to manipulate highly pathogenic strains of avian influenza from the H5 or H7 subtype to arrive at a strain that is transmissible in mammals (typically ferrets). Many of these studies also test the resulting strains for pathogenicity. The studies of interest here use a variety of methods including:

- Simple serial passaging in ferrets, and
- Directed, sequential reassortment of an avian-origin strain with a pandemic strain.

In the serial passaging experiment, the researchers claim that no loss of pathogenicity is observed compared to the highly pathogenic parental strain after ten passages in ferrets. However, too few animals were used to assess pathogenicity to detect some loss of pathogenicity. In the reassortment study, the authors show that two of the four transmissible strains they identified are *more* pathogenic than either

[470] For example, Natalia A. Ilyushina et al., "Adaptation of Pandemic H1N1 Influenza Viruses in Mice," *Journal of Virology* 84 no. 17 (September 2010): 8607-8616.

parental strain (although they tested this phenotype in mice). Although the authors make this claim, their experimental design may not have been sufficient to detect true differences in pathogenicity from the parental strain relevant to humans.

The serial passaging experiment could be repeated by an actor with limited skills in molecular biology to obtain a strain of avian influenza that is newly transmissible in mammals and highly pathogenic (albeit this actor would need animals and the facilities to contain them). Moreover, all investigators characterized the resulting strains and published enough information (in the initial or follow-on papers) so that the strain of the desired characteristics could be directly produced by reverse genetic methods. Specifically, in the case where serial passaging was used, the mutations acquired by the strains of interest were published. In the case of the reassortment study, the gene segments that contributed to the transmissible strains from both parental strains were published.

For this reason, the GoF information risk for enhanced transmissibility while maintaining pathogenicity of influenza viruses is already realized. Further publication on this topic would likely not exacerbate this risk because existing published methods are relatively simple to replicate. Actors skilled in reverse genetics could instead recreate the strains with the desired traits that these groups characterized.

> Two groups have published simple methods to increase the transmissibility in mammals of pathogenic strains of influenza that were previously transmissible only in avians while maintaining (or enhancing) pathogenicity. Therefore, this type of information risk is already realized.

As discussed in the enhanced transmissibility section above, our retrospective study of the literature cannot conclusively determine the dual utility of these publications. Further studies with more relevant model animals would provide useful information about whether the resulting strains are likely to be both more pathogenic and highly transmissible in humans. Also, our study (and any animal study) is unlikely to conclusively determine if a transmissible strain would be similarly transmissible to seasonal influenza in human populations.

8.5.1.4 Overcoming Natural or Induced Immunity

As discussed above, the evasion of immunity is a desirable trait for a malicious actor if they wish to use a strain of influenza that recently circulated (and, therefore, need to overcome the significant residual immunity that exists in the population). For this reason, the information risk related to the ability of a modified strain to overcome natural or induced immunity inheres in the *methods* to foster an antigenic change in any desired virus strain. Therefore, methods published using attenuated strains are still relevant to this information risk. All of the published papers reviewed in this study focus on elucidating the mechanisms by which new strains with different antigenic profiles evolve. The methods involved include:

- Identification of unique changes between a parental strain and an antigenically distinct strain, followed by the introduction of these unique changes into the parental strain by reverse genetics,
- Serial passaging in cells in the presence of neutralizing antibodies, and
- Serial passaging in immunized animals.

The serial passaging experiments could be repeated by an actor with limited skills in molecular biology to obtain an antigenically distinct strain of influenza. Although one of the studies resulted in antigenically distinct strains that are as pathogenic (or even more so, in mice) than the parental stains, other studies that

use large numbers of passages create significantly attenuated strains and still others do not characterize the pathogenicity of their strains (however, fitness was assessed and maintained). Some studies using reverse genetics produce strains that are antigenically distinct and pathogenic. No matter which method was used, all investigators in the reviewed studies characterized the resulting strains and published enough information (in the initial or follow-on papers) so that the strain with the desired characteristics (distinct antigenicity and pathogenicity) could be directly produced by reverse genetic methods by a malicious actor with significant skill in molecular biology.

For this reason, the GoF information risk for creating strains of influenza that are antigenically distinct from their parental strain is already realized. Further publication on this topic would likely not exacerbate this risk because existing published methods are relatively simple to replicate.

> Several groups have published simple methods to create antigenically distinct strains of influenza virus. Therefore this type of information risk is already realized. To our knowledge, no one has published methods to create strains of influenza virus able to overcome the protection afforded by vaccination in general, which poses a remaining information risk.

No papers were found that describe the development of influenza strains able to overcome protective immunity without creating an antigenically distinct virus. Methods have been published describing the use of other pathogens (viruses and bacteria) expressing exogenous factors to create strains that enable the pathogen to kill infected hosts despite being effectively immunized with a vaccine matched to the serotype of the pathogen. However, these pathogens have larger and more plastic genomes than the influenza viruses and therefore, the development of such a strain of influenza would be a major research undertaking. Moreover, the benefits of this type of research would be highly suspect. Regardless of the reasons not to perform this research, the publication of methods to produce strains of influenza able to overcome immunity regardless of its serotype would pose an information risk.

8.5.1.5 Evasion of Diagnostics

Current generation influenza diagnostics function either via the recognition of epitopes in the virus (or antibodies to these antigens generated by a patient) or via recognition of unique sequences in the genome of the virus. Diagnostics that function by leveraging antibodies could be evaded in much the same way that host immunity can be evaded, so this possibility will not be discussed further.

No papers reviewed in this study discussed the production of strains of influenza that can evade diagnosis by the alteration of its genetic makeup (except for changes in the genome that lead to changes in antigens). Actors with skills in molecular biology (and knowledge of the genetic targets of the assays) could create strains of viruses with a series of silent mutations (mutations that alter the genomic material but do not change the encoded proteins) to confound recognition. However, codon usage in viruses is sometimes tightly linked to fitness (or other desired traits), and therefore, a malicious actor must test their new strains to ensure that all desired phenotypes were not lost. In any case, the publication of methods that demonstrate how to evade diagnostics via the alteration of the genome pose a remaining information risk.[471]

> Several groups have published simple methods to create antigenically distinct strains of influenza virus which can evade diagnostics that use the antigenic properties of the virus. Therefore, this type of information risk has already been realized. No methods have been published describing the modification of influenza strains to evade diagnostic methods reliant on unique genomic signatures. Therefore, this type of information risk remains.

[471] Wong E, et al. (2010) "Codon usage bias and the evolution of influenza A viruses," *BMC Evolutionary Biology* 10, 253

8.5.1.6 Antiviral Resistance

Because the creation of antimicrobial resistant strains is part of the drug-development process and part of risk assessment process for determining when the effectiveness of antimicrobials may expire, several publications on the creation of influenza strains resistant to antivirals could be found. The methods used involved:

- Identification of unique changes between a parental strain and a drug resistant strain, followed by the introduction of these unique changes into the parental strain by reverse genetics.
- Serial passaging in cells in the presence of low concentrations of the antiviral, and
- Infection of animals treated with sub-optimal concentrations of the antiviral.

Serial passaging and *in vivo* experiments could be repeated by an actor with limited skills in molecular biology to obtain an antiviral-resistant strain of influenza. *In vivo* methods also simultaneously select for fitness (and in some cases, pathogenicity), suggesting that the resulting strains might still be useful in a weapon. Studies using reverse genetics produce strains with several mutations, some of which lead to antiviral resistance and others compensate for the defects in pathogenicity or fitness caused by the primary mutation. These investigators characterized the resulting strains and published enough information so that the strain with the desired characteristics (antiviral resistance and pathogenicity) could be directly produced by reverse genetic methods by a malicious actor with significant skill in molecular biology.

For this reason, the GoF information risk for creating strains of influenza that are antiviral resistant is already realized. Further publication on this topic would likely not exacerbate this risk because existing published methods are relatively simple to replicate.

> Several groups have published simple methods to create antiviral resistant strains of influenza. Therefore this type of information risk is already realized.

8.5.1.7 Increased Production in Cell Culture or Eggs

The vast majority of studies identified in which the production of viruses in cell culture or eggs was enhanced involved the introduction of some components of pathogenic strains into attenuated strains. These studies were designed to create attenuated strains with the immunoreactive antigens of the pathogenic strains suitable for use as vaccines. Several studies discuss the generation of strains with enhanced growth properties by reassorting pathogenic influenza viruses with attenuated strains adapted for growth in eggs or culture.[472] However, these strains were chosen because they simply express the HA and NA antigens in a virus suitable for vaccination.

Others have adapted attenuated strains to achieve a 100-fold increase in titer after growth in cell culture by serial passaging, but this method is likely to allow pathogenicity and transmissibility to drift.[473] Others

[472] Zhang W et al. (2011) "Increase in viral yield in eggs and MDCK cells of reassortant H5N1 vaccine candidate viruses caused by insertion of 38 amino acids into the NA stalk," *Vaccine* 29, vol 45: 8032-41.

[473] For example: Murakami S., Horimoto T., Ito M., Takano R., Katsura H., Shimojima M., Kawaoka Y., "Enhanced growth of influenza vaccine seed viruses in vero cells mediated by broadening the optimal pH range for virus membrane fusion," *Journal of Virology* 86 (2012): 1405-1410.

have used random mutagenesis of the HA gene to identify high growth mutants, but these mutants are likely to be poorly pathogenic.[474]

In contrast to these studies, other researchers have serendipitously found that some pathogenic strains demonstrate increased growth in cells or eggs using a variety of methods, including:

- Serial passaging in animals, and
- Identification of unique changes between a parental strain and a highly pathogenic strain, followed by the introduction of these unique changes into the parental strain by reverse genetics

The researchers leveraging reverse genetics introduced changes found in a highly pathogenic strain into a less pathogenic background to determine if these changes were sufficient to increase pathogenicity. They serendipitously, but perhaps not surprisingly, found that the growth of these pathogenic virus strains in culture or *in vivo* was enhanced. Since a malicious actor would likely desire this combination of phenotypes, this type of study generates biosecurity information risk. However, beyond using reverse genetics to synthesize these strains, these pathogenic, high-titer strains could be directly acquired from clinical samples.

> Several groups have published approaches of developing highly pathogenic strains of influenza that grow to a higher titer than their parental strains. Repeating these experiments requires skills in molecular biology. Those without molecular biology skills could repeat a serial passaging experiment, but they must take measures to retain pathogenicity of the final strain. For these reasons, little information risk remains for this trait.

Actors unable to acquire the specific highly pathogenic strains studied and who lack skills in molecular biology skill could instead repeat one of the serial passaging studies published. However, both of these studies used mice as their model system to identify strains that were more pathogenic and grew to a higher titer in cell culture or in eggs. Because of the weakness of the mouse animal model, passaging in mice may not lead to strains that are pathogenic in people. Presumably, passaging the virus in another animal model would retain the virulence of the strain (as shown in the passaging experiments above) and may result in higher growth variants.

8.5.2 State of the Science of GoF Experiments in the Coronaviruses

8.5.2.1 Enhanced Transmissibility in Mammals

No model system currently exists for the study of transmissibility of SARS- or MERS-CoV and, therefore, no studies have described methods to increase the transmissibility of these viruses. Therefore a significant information risk remains for any studies that describe the development of an animal model of transmission. However, these studies are necessary to understand the evolution of the viruses, their life cycle, their associated pathology, and pathways for developing vaccines and drugs.

[474] Ye J. et al. (2015) "Error-prone pcr-based mutagenesis strategy for rapidly generating high-yield influenza vaccine candidates," *Virology* 482: 234-243.

8.5.2.2 Enhanced Pathogenicity

SARS- and MERS-CoV do not normally infect mice, so the virus must be manipulated to infect this host to study pathogenicity. In fact, mouse-adapted SARS-CoV cannot effectively bind to or infect human cells. For this reason, although several groups described strains of coronaviruses that have enhanced pathogenicity compared to the parental, mouse-adapted coronavirus strains, these viruses are presumably acting in mice more like the wild type SARS- and MERS-CoV do in humans. These experiments are performed to learn how SARS- and MERS-CoV became pathogenic to humans and not to determine how they could become more pathogenic in the future. If new experimental systems were developed, an information risk would be possible. That being said, even if these experiments were to be conducted, very little room for the enhancement of pathogenicity is possible due to the high case-fatality rate and severity of the symptoms of the diseases these pathogens cause.

> Model animal systems for the study of SARS- or MERS-CoV can give us information on how these pathogens became dangerous to humans but probably not how they can become more dangerous. If new experimental systems were developed, an information risk would be possible.

8.5.2.3 Enhanced Transmissibility While Maintaining/Enhancing Pathogenicity

Currently, no model system for the study of transmissibility of SARS- or MERS-CoV exists and, therefore, no studies have described methods to increase the transmissibility of these viruses. Therefore a significant information risk remains.

8.5.2.4 Evading Diagnostics

Current generation coronavirus diagnostics function either via the recognition of epitopes in the virus (or antibodies generated against these antigens by a patient) or via recognition of unique sequences in the genome of the virus. Diagnostics that function by leveraging antibodies could be evaded in much the same way that host immunity can be evaded. We found two papers that describe the selection of a SARS-CoV that can escape binding by antibodies. In these papers, researchers serially passaged the virus in cells in the presence of antibodies derived from infected patients. In one study, the researchers studied how pathogenic the escape mutants were and found that some retained their pathogenicity.

No papers reviewed described the production of strains of coronaviruses that can evade diagnosis by the alteration of its genetic makeup (except for changes in the genome that lead to changes in antigens). Actors with skills in molecular biology (and knowledge of the targets of the assays) could create strains of viruses with a series of silent mutations (mutations that alter the genomic material but do not change the encoded proteins) to confound recognition. However, codon usage in viruses is sometimes tightly linked to fitness (or other desired traits).[475] For this reason, a malicious actor must test their new strains to ensure that the desired phenotypes were not lost. The publication of methods that demonstrate how to evade diagnostics via the alteration of the viral genome pose a remaining information risk.

> We found two groups that have published simple methods to create antigenically distinct strains of SARS-CoV which can evade diagnostics that use the antigenic properties of the virus. Therefore, this type of information risk has already been realized. No methods have been published describing the modification of coronavirus strains to evade diagnostic methods that target genomic sequence. Therefore, this type of information risk remains.

[475] Gu W. et al. (2004) "Analysis of synonymous codon usage in SARS Coronavirus and other viruses in the Nidovirales." *Virus Research* 101, vol 2: 155-161.

8.5.2.5 Overcoming Countermeasures and Immunity

As no approved vaccines or antivirals exist for prevention or treatment of infections caused by the coronaviruses, experiments of this type are not possible so they pose no information risk. Moreover, methods to evade antibody-based countermeasures and small molecule countermeasures are well established for other pathogens, including influenza. If such countermeasures were developed in the future, an adversary is likely able to leverage any of these methods to develop strains of the coronaviruses that are resistant to the countermeasures.

Moreover, infections by the SARS and MERS coronaviruses are sufficiently rare so that an adversary has no need to create a strain that can evade residual immunity from past infections.

8.5.2.6 Increased Production in Cell Culture or Eggs

We found no papers describing the enhancement of viral growth in culture or eggs. However, SARS- and MERS-CoV grow to a relatively high titer in culture, suggesting enhancement of this trait is unnecessary.

8.5.3 Overview of the State of the Science of GoF Experiments

Figure 8.3, below summarizes the analysis of the literature already published relevant to GoF research. We found that much of the dual-use information that could arise from GoF experiments has already been published for the influenza viruses. For this reason, much of the information risk for this pathogen has already been realized. The remaining information risk inheres in the creation of simpler experimental approaches to the development of strains with enhanced pathogenicity or enhanced growth, and any method that leads to the creation of strains that can avoid protection by *any* vaccine or the evasion of diagnostics via alteration of its genome. In contrast, the lack of model animal systems for the study of transmission or enhanced pathogenicity of the coronaviruses leaves a significant information risk if these systems were developed. That is, future experiments that describe how to make a strain of the coronaviruses more contagious (or more deadly) have a significant information risk. Although medical countermeasures for the coronaviruses do not yet exist, if they were developed a malicious actor could easily leverage simple experimental procedures published for other pathogens to create strains that overcome the countermeasures.

Dual-Use GoF Phenotype	Influenza	Coronaviruses
Enhanced transmissibility in mammals		
Enhanced pathogenicity in mammals	Published methods require skills in molecular biology. No publications exist on creation of influenza strains that lead to chronic illness.	
Enhanced transmissibility while maintaining pathogenicity		
Overcoming natural or induced immunity	Via the creation of antigenically distinct strains only	N/A
Evading diagnostics	Evasion of immunological diagnostics only	Evasion of immunological diagnostics only
Antiviral resistance		N/A
Enhanced production in cell culture or eggs	Published methods require skills in molecular biology.	N/A

Figure 8.3. Status of the publication of potentially dual-use information relevant to GoF research. White denotes that no significant information risk is left either because the relevant information has already been published or the resulting trait is not dual-use. Dark shading denotes a lack of publications on the topic so a significant information risk may remain. Grey denotes that some information risk may be remaining.

8.6 Evaluation of the Capability and Intent of Malicious Actors to Leverage Dual Use Information

Given the analysis above, non-unique information risk resides in the ability to create strains of highly-transmissible, antiviral resistant pathogens that have a high case fatality rate and are not limited by residual immunity brought about by the natural circulation of similar strains. That being said, this risk is already realized because the methods to produce these strains is already published. For malicious actors interested in developing a uniquely capable incapacitating agent, research on the development of influenza strains that produce chronic or long-lasting illness would be of interest.

We acknowledge that GoF research is just one means through which a malicious actor may obtain such a pathogen. By leveraging GoF information, sophisticated actors could use reverse genetics to create a strain that was previously described by other researchers with all desired characteristics. Actors with limited skills in molecular biology could use the selection experiments described in the literature to attain strains that may have all the desired traits, but extensive testing would be necessary to identify a strain with all such characteristics. This section describes the actors with the capability and motivation to seek to leverage this information.

State actors, who in the past have sought deadly strains and incapacitating strains of pathogens for use in offensive weapons programs, clearly often have the ability to acquire the equipment and expertise to use reverse genetics to create any strain of influenza or coronavirus described in the literature. Moreover, states likely would have the ability to design and produce a cache of vaccines (in the case of modified influenza viruses) that could protect their own population from the contagion that may spread from their intended target (and to protect their workers during the development and weaponization process). For this reason, if states were to leverage GoF information for malicious use, they would likely target information

on the influenza viruses (instead of the coronaviruses) and would be uninterested in strains that can avoid any type of vaccine protection. That being said, secrecy inside a state program may hamper the coordination of the offensive and defensive components of a biological weapons program. For example, those working on countermeasures in the Soviet biological weapons program lacked the clearance to know about the offensive side.[476,477] Moreover, the stockpiling of vaccines specific to a strain of influenza virus that has no risk of a natural outbreak could undesirably broadcast information about a state's offensive program.

Other malicious actors, from individuals to terrorist groups, have so far shown very little ability to acquire or grow biological agents. The few terrorist groups that have attempted to do so have relied on bacterial agents, not viral agents. The publically available literature suggests that no sub-state group has so far demonstrated the scientific sophistication and the resources necessary to leverage GoF information for malicious purposes.

In theory, a lone outsider (or small group of outsiders) with scientific training may have the ability to perform the manipulations necessary to obtain modified pathogens via relatively simple methods. Alternatively, if unconstrained by time and resources these technically trained actors may be able to use reverse genetics to obtain any desired strain. Industry standards for customer and sequence screening, in part supported by US government guidance, may prevent actors from acquiring synthesized pathogens via genetic material synthesized by industry. However, some gene synthesis (domestically and internationally) companies do not voluntarily follow the industry standards or US government guidance.

Lone scientific actors working in GoF laboratories (i.e., insiders) can simply directly acquire the desired strains as described in Chapter 7, above. Given the access controls implemented in containment laboratory, there is little opportunity for an insider to carry out a clandestine development and testing program inside the laboratory they legitimately have access to. Therefore, insiders are not considered a driver of information risk because they have access to strains and unpublished data.

A malicious actor developing an engineered strain would presumably need the resources to test the newly developed strain to ensure that it has all the traits desired. Animal testing facilities are expensive and difficult to covertly obtain, establish and operate without running the risk of self-infection and/or exposing the public. Sub-state groups, however, are likely to be satisfied with more rudimentary animal testing than those conducted by scientists seeking to publish in a peer-reviewed publication.[478]

> Of the potential malicious actors, only state actors have the resources, technical sophistication and motivation to leverage dual-use information arising from GoF studies. These states could protect their own population by secretly stockpiling an effective vaccine against their modified agent, suggesting they may prefer to target influenza viruses over the coronaviruses.

If somehow a scientific actor can create a facility that is sufficiently remote to develop the strains and perform the needed testing without being noticed (by intelligence gathering or by causing an outbreak), they could produce a highly contagious, highly lethal strain of virus. These properties avoid an often noted shortcoming of small groups wishing to produce an effective biological weapon: that they lack either the scientific expertise to create a useful biological agent or the engineering expertise to create a useful biological munition (both of which are normally needed to create a weapon capable of inflicting

476 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.

477 Ouagrham-Gormleym S (2014) *Barriers to Bioweapons Ithaca:* Cornell University Press.

478 Danzig R, et. al. (2012) "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," Center for a New American Security http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf.

mass casualties). Infections caused by rudimentary devices (like a spray bottle) may be enough to seed a global pandemic causing mass casualties. However, as noted in Chapter 7, above, we know of no lone actor who has ever desired to cause a global pandemic (albeit, a small group, R.I.S.E, did). Moreover, terrorist organizations and individuals appear to act more on the emotional and societal motivations towards radicalization and violence, suggesting a greater focus on the immediate, rather than long-term, response or outcome of an act.[479] Of course, historical examples do exist of individuals and terrorist groups that invested significant resources and time in planning of an attack (see Section 7). Whether the calculus of long-term illness or mass casualty through infection is attractive to sub-state actors is not known from publicly available literature.

One final caveat: the unsophisticated actor today could leverage advancing technologies to gain a significant body of skills and knowledge. Software is being developed to automate the research process, with the possible outcomes of increasing reproducibility and decreasing human involvement in the experimental process.[480] Finally, several online blogs, websites, video journals, and analytic technologies increasingly are being used to identify experimental protocols, troubleshoot experimental problems, and design experimental reagents such as DNA primers for polymerase chain reaction experiments.[481] Together, these changes may increase democratization of life sciences experiments and lower the level of skill and advanced scientific knowledge needed to conduct experimental procedures.[482]

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[479] De Angelis T (2009) "Understanding Terrorism," American Psychological Association. http://www.apa.org/monitor/2009/11/terrorism.aspx. Accessed on October 27, 2015.

[480] For example, see Amyris Genome Compiler. Accessible at http://www.genomecompiler.com/amyris-dna-construction-on-genome-compiler/?utm_source=referral_website&utm_medium=press_release&utm_term=5_8_2015&utm_content=website&utm_campaign=amyris_alpha_program. Accessed on September 12, 2015.

[481] For example, see JOVE. Accessible at http://www.jove.com/. Accessed on September 12, 2015.

[482] For additional discussion, J Revill and C Jefferson. Tacit knowledge and the biological weapons regime. Sci Pub Policy. 2013; KMVogel. Phantom Menace or Looming Danger? A New Framework for Assessing Bioweapons Threats. 2013. The Johns Hopkins University Press. (Baltimore, Maryland).

9 Benefit Assessment of GoF Research TEST

9.1 Overview of Results

The Benefit Assessment evaluated the potential benefits of GoF experimental approaches involving coronaviruses (CoVs) and influenza viruses to scientific knowledge and public health. Public health benefits included benefits to biosurveillance, to the development of medical countermeasures (MCMs), and to decision-making in public health policy. In each case, the ability of GoF approaches to address gaps in scientific knowledge or shortcomings in public health was compared to the ability of alternative approaches to address those same gaps, which enabled identification of the unique benefits of GoF research. Two types of alt-GoF approaches were considered: alternative experimental approaches that can provide the same or similar information and alternative scientific or technical innovations that can address the same public health gaps through completely different mechanisms. Of note, unlike the risk assessment, the benefit assessment was limited to the evaluation of GoF approaches that have been described in the scientific literature.

Within the field of CoV research, GoF approaches in the following phenotypic categories were identified: enhanced pathogen production, altered host range, enhanced virulence, and evasion of therapeutics in development. Within the field of influenza research, GoF approaches in the following phenotypic categories were identified: enhanced virus production, mammalian adaptation and enhanced transmissibility, enhanced virulence, evasion of vaccines or therapeutics, and evasion of existing natural or induced adaptive immunity. The following figure summarizes the results of the benefit assessment.

GoF Phenotype	Coronaviruses	Seasonal Influenza Viruses	Animal Influenza Viruses	Pathogenic Reassortant Influenza Viruses*
Adaptation to mammals				
Enhanced transmissibility	N/A			
Enhanced pathogenicity				
Evasion of vaccines in development	N/A			
Evasion of existing natural or induced adaptive immunity	N/A		N/A	N/A
Evasion of therapeutics				
Enhanced virus production				
Reassortment (multiple GoF phenotypes possible)	N/A			

**Pathogenic reassortants influenza viruses include reassortants comprised of gene segments from seasonal and pandemic or seasonal and animal influenza viruses.*

Figure 9.1 Summary of the benefits of GoF research by phenotype. White indicates that the phenotypic change cannot be achieved or is not relevant (given the current state of model systems, the current state of MCMs, or the biological characteristics of the virus). Dark grey indicates that the current phenotypic change may be achievable but has not been undertaken in the scientific literature. Light grey indicates that the approach provides unique benefits to scientific knowledge and/or public health. Medium grey indicates that the benefits of GoF approaches and alternative approaches are overlapping; that is, that alt-GoF approaches can address the same scientific knowledge or public health gaps that GoF approaches can address. Note that medium grey does not indicate that GoF and alt-GoF approaches are equally capable of addressing those

gaps, simply that a more nuanced evaluation is needed to understand the relative value of GoF and alt-GoF approaches.

The brief section that follows provides an overview of the GoF benefits identified in each phenotypic category.

9.1.1 Coronaviruses

GoF approaches that alter host range and enhance virulence uniquely enable the development of animal model systems that recapitulate human disease pathogenesis, which are critical for the study of CoV pathogenesis and for establishing the safety and efficacy of candidate vaccines and therapeutics. This manipulation to a new host typically attenuates virulence in the original host (in the case of SARS and MERS-CoV, humans). GoF approaches that enhance virulence are also uniquely capable of demonstrating that live attenuated vaccines (LAVs) do not recover virulence upon growth *in vivo*, an important aspect of safety testing of candidate LAVs. Of note, this particular approach simply increases the human health risk of the attenuated strain to approach that of wild type strains. GoF approaches that enhance virulence represent the most efficient and effective strategy for discovering novel virulence factors, which may be good targets for new therapeutics. However, several alternative strategies for the development of new therapeutics are being actively pursued and have also shown promise. GoF approaches that lead to evasion of therapeutics in development are critical for the development and regulatory approval of new therapeutics. Because these therapeutics are not yet widely available, no increase in human health risk is posed by resistant strains. GoF approaches that alter host range and enhance virulence provide unique benefits to study cross-species adaptation and pathogenicity, but alternative approaches may also be used.

9.1.2 Influenza Viruses

Across all GoF phenotypes, GoF approaches provide unique benefits to the study of the mechanistic basis of the phenotype under study as well as the evolutionary mechanisms driving acquisition of that trait, though alternative approaches may also be used. Alternative approaches have stringent limitations for the study of mechanisms underlying mammalian transmissibility of animal influenza viruses, as animal flu viruses that efficiently transmit in humans do not exist in nature.

GoF approaches that enhance virus production are uniquely critical for their current ability to produce sufficient and timely influenza vaccines for seasonal flu epidemics and flu pandemics; they represent the only strategy for improving existing vaccine production capabilities in the near-term. Of note, these particular approaches attenuate an otherwise pathogenic strain while enhancing its growth properties.

GoF approaches that enhance the infectivity, transmissibility, and virulence of animal flu viruses inform pandemic risk assessments of circulating influenza viruses, which guide downstream decision-making about investments in pre-pandemic vaccine development and other pandemic preparedness initiatives. Specifically, GoF approaches are uniquely critical for strengthening the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence, which can be used to infer phenotype from sequence for the risk assessment. In general, molecular marker data moderately contribute to the overall risk associated with a particular virus. However, molecular marker data play an important role in rapid risk assessments when novel flu viruses first emerge in human populations due to the early availability of viral sequence data. These risk assessments facilitate more rapid initiation of response activities such as pre-pandemic vaccine development. Of note, realization of these benefits is subject to significant advancements in the state of knowledge about mechanisms underlying mammalian adaptation,

transmissibility, and virulence, as well as expansion of sequencing capabilities across public health laboratories involved in influenza surveillance.

GoF approaches that enhance the infectivity and virulence of influenza viruses are also used to develop animal models that support the study of disease pathogenesis and medical countermeasure (MCM) development. GoF approaches that lead to evasion of therapeutics in development are critical for the development and regulatory approval of new therapeutics. Of note, the acquisition of resistance to novel classes of therapeutics is not expected to confer cross-resistance to existing antivirals (i.e., adamantanes or neuraminidase inhibitors). Thus, when these experiments involve drug candidates within new classes of therapeutics, which are not yet widely available, no increase in human health risk is posed by resistant strains. However, similar approaches using licensed therapeutics inform therapeutic recommendations for seasonal influenza infections and pandemic preparedness initiatives for high-risk animal influenza viruses, but phenotypic approaches for antiviral sensitivity testing are also used for these purposes. GoF approaches that lead to evasion of vaccines are uniquely capable of determining whether viruses can acquire mutations to escape neutralization of candidate broad-spectrum or universal influenza vaccines, a critical aspect of testing the potential field efficacy of vaccines in development. Most of these experiments involve next-generation influenza vaccine candidates targeting epitopes other than the globular head domain of the hemagglutinin (HA) protein, the target of current influenza vaccines. Given that the globular head domain of HA is the immunodominant protein of influenza viruses and that these next-generation vaccines are not yet widely available, strains that can overcome the protection afforded by these vaccines are expected to pose a minimal increase in human health risk relative to wild type strains. GoF approaches that lead to evasion of existing natural or induced immunity have potential to improve the efficacy of seasonal influenza vaccines, but this benefit is subject to advancements in the state of knowledge about the mechanistic basis of antigenic drift as well as expansion of sequencing capabilities across public health laboratories involved in global influenza surveillance. Finally, GoF studies involving reassortment, which may lead to one or more phenotypic changes, are uniquely capable of providing information that can be used to prioritize community-level interventions aiming to prevent opportunities for co-infections that could lead to the generation of reassortant viruses with phenotypic properties of concern.

9.1.3 Summary

Chapter 9 provides a relatively brief description of all of the benefits of GoF research that were identified in this study. Chapter 15 provides a fully referenced and in-depth discussion of these findings and includes a summary table for each GoF benefit, which describes the relative strengths and limitations of GoF and alt-GoF approaches that can achieve that benefit. As the relative ability of a given GoF (or alt-GoF) approach to address a particular scientific knowledge or public health gap often hinges on nuanced differences between the benefits and limitations of different approaches, readers who seek an in-depth understanding of the benefits of GoF research are directed to chapter 15.

The following table summarizes the set of benefits identified for each GoF phenotype and directs readers to the relevant sections and summary tables that accompany each benefit.

Table 9.1. List of Potential Benefits of GoF Research Involving Coronaviruses

Benefit Category	Potential Benefit	Unique Benefit?*	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Enhanced virus production						
Scientific Knowledge	Develop *in vitro* model systems for studying bat CoVs	Partial	9.3.5.1.1; 15.1.4.1	15.3	Immediate	N/A
Altered host range (mammalian adaptation)						
Scientific Knowledge	Gain insight into mechanistic basis of cross-species adaptation	Partial	9.3.5.1.1; 15.1.3.1	15.1	Immediate	N/A
Scientific Knowledge	Develop *in vitro* model systems for studying bat CoVs	Partial	9.3.5.1.1; 15.1.4.1	15.3	Immediate	N/A
Scientific Knowledge	Develop animal models for studying CoV pathogenesis	Partial	9.3.5.1.3; 15.1.4.2	15.4	Immediate	N/A
Vaccines and therapeutics	Develop animal models for testing candidate MCMs	Partial	9.3.5.4.1; 15.1.4.2	15.4	Long term	Development and licensing of new MCMs is a long process
Vaccines and therapeutics	Develop model system for testing broad-spectrum efficacy of MCMs	Partial	9.3.5.4.2; 15.1.5.3	15.8	Long term	Development and licensing of new MCMs is a long process
Enhanced virulence						
Scientific Knowledge	Gain insight into mechanistic basis of CoV virulence	Partial	9.3.5.1.2; 15.1.3.2	15.2	Immediate	N/A
Scientific Knowledge	Develop animal models for studying CoV pathogenesis	Partial	9.3.5.1.3; 15.1.4.2	15.4	Immediate	N/A
Vaccines and therapeutics	Develop animal models for testing candidate MCMs	Partial	9.3.5.4.1; 15.1.4.2	15.4	Long term	Development and licensing of new MCMs is a long process

Table 9.1. List of Potential Benefits of GoF Research Involving Coronaviruses

Benefit Category	Potential Benefit	Unique Benefit?*	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Therapeutics	Identify new therapeutic targets	Partial	9.3.5.3.1; 15.1.5.2	15.6	Long term	Development and licensing of new therapeutics is a long process
Vaccines	Support development of live attenuated vaccines	Yes	9.3.5.2; 15.1.5.1.1	15.5	Long term	Development and licensing of new vaccines is a long process
Evasion of therapeutics in development						
Therapeutics	Gain insight into therapeutic's mechanism of action	Partial	9.3.5.3.2; 15.1.5.2.2.	15.7	Long term	Development and licensing of new therapeutics is a long process
Therapeutics	Facilitate regulatory approval of new therapeutics	Yes	9.3.5.3.2; 15.1.5.2.3	N/A	Long term	Development and licensing of new therapeutics is a long process
Therapeutics	Inform development of therapeutic strategies that minimize development of resistance	Yes	9.3.5.3.3; 15.1.5.2.4	N/A	Long term	Development and licensing of new therapeutics is a long process

**The "Unique Benefit" column indicates whether the benefit indicated in the previous column is unique or whether alt-GoF approaches can achieve the same general benefit. "No" indicates that alt-GoF approaches can provide nearly identical benefits, with respect to the quality, scope, and timeliness of the benefit; "Yes" indicates that alt-GoF approaches cannot provide the same benefit; and "Partial" indicates that alt-GoF approaches can provide similar benefits but may be limited in some way when compared to the GoF approach. Of note, a "Partial" entry does not indicate that the potential benefits of GoF and alt-GoF approaches are the same but rather that a more nuanced evaluation is needed to understand the relative benefits of GoF and alt-GoF approaches.*

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses

Agent (Influenza Virus Strains)*	Benefit Category	Potential Benefit	Unique Benefit?**	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Enhanced virus production							
Seasonal, animal	Scientific Knowledge	Gain insight into the mechanistic basis of high growth of vaccine viruses	Partial	9.5.5.1; 15.2.4.2	15.10	Immediate	N/A
Seasonal, animal	Vaccines	Enable the sufficient and timely production of flu vaccines	Yes	9.5.5.2.1; 15.2.3	15.9	Immediate	Status quo – GoF approaches are currently a key aspect of flu vaccine production
Seasonal, animal	Vaccines	Shorten future vaccine production timelines	Partial	9.5.5.2.2; 9.5.5.2.3; 15.2.4.3	15.11; 15.12; 15.13	Near term	GoF insights can be applied to vaccine production without the need for FDA approval
Mammalian adaptation and enhanced transmissibility							
Animal	Scientific Knowledge	Gain insight into mechanistic basis of mammalian adaptation and acquisition of transmissibility	Partial	15.3.3	15.14; 15.15; 15.16; 15.17	Immediate	N/A
Animal	Surveillance	Inform surveillance of circulating animal flu viruses by enabling sequence-based prediction of adaptation and transmissibility	Partial	9.6.5.2; 15.3.4	15.18	Near to long term	Information from GoF studies can be immediately applied to surveillance and downstream decision-making about pandemic preparedness activities, including pre-pandemic
Animal	Policy	Inform pandemic risk assessment of animal flu viruses and downstream decision-making about investments in pandemic preparedness activities	Partial	9.6.5.3; 15.3.5.2	15.19	Near to long term	

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses

Agent (Influenza Virus Strains)*	Benefit Category	Potential Benefit	Unique Benefit?**	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Animal	Policy, Vaccines	Guide selection of strains for pre-pandemic vaccine development	Yes	9.6.5.3; 15.3.5.2.5.1	15.4	Near term to long term	vaccine development. However, this benefit is constrained by scientific uncertainties associated with the data. The benefit of GoF information to surveillance is expected to increase over time, as the state of the science advances.
Enhanced virulence							
Seasonal, animal	Scientific Knowledge	Gain insight into mechanistic basis of influenza virulence	Partial	9.7.5.1; 15.4.3.1; 15.4.3.2	15.20; 15.21; 15.22	Immediate	N/A
Seasonal, animal	Scientific Knowledge	Develop animal models for studying influenza pathogenesis	Partial	9.7.5.1.3; 15.4.3.3	15.23	Immediate	N/A
Animal	Surveillance	Inform surveillance of circulating animal flu viruses by enabling sequence-based prediction of virulence	Partial	9.6.5.2; 15.3.4	15.18	Near to long term	Information from GoF studies can be immediately applied to surveillance and downstream decision-making about pandemic preparedness activities, including
Animal	Policy	Inform pandemic risk assessment of animal flu viruses and downstream decision-making about investments in pandemic preparedness activities	Partial	9.6.5.3; 15.3.5.2	15.19	Near to long term	

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses

Agent (Influenza Virus Strains)*	Benefit Category	Potential Benefit	Unique Benefit?**	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Animal	Policy, Vaccines	Guide selection of strains for pre-pandemic vaccine development	Yes	9.6.5.3; 15.3.5.2.5.1	15.4	Near to long term	pre-pandemic vaccine development. However, this benefit is constrained by scientific uncertainties associated with the data. The benefit of GoF information to surveillance is expected to increase over time, as the state of the science advances.
Seasonal, animal	Vaccines	Support development of live attenuated vaccines	Yes	9.7.5.3.1; 15.4.5.1	N/A	Long term	Development and licensing of new vaccines is a long process
Animal	Vaccines	Improve safety of vaccine production process by identifying virulence markers that can be removed from vaccine viruses	Partial	9.7.5.3.2; 15.4.5.2	15.24	Intermediate term	FDA approval may be needed for application of GoF insights to vaccine production
Seasonal, animal	Therapeutics	Identify new therapeutic targets	Partial	9.7.5.4; 15.4.5.3	15.25	Long term	Development and licensing of new therapeutics is a long process

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses

Agent (Influenza Virus Strains)*	Benefit Category	Potential Benefit	Unique Benefit?**	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Seasonal, animal	MCMs	Develop animal models testing candidate MCMs	Partial	9.7.5.5; 15.4.3.3	15.23	Long term	Development and licensing of new MCMs is a long process
Evasion of existing natural or induced immunity							
Seasonal, pandemic***	Scientific Knowledge	Gain insight into mechanistic basis of antigenic drift	Partial	9.8.5.1; 15.5.3	15.26; 15.27; 15.28	Immediate	N/A
Seasonal	Surveillance	Improve antigenic surveillance by enabling sequence-based prediction of antigenic phenotype	Partial	9.8.5.2; 15.5.4	15.29	Near to long term	Information from GoF studies can be immediately applied to surveillance and downstream strain selection for seasonal flu vaccines, but that benefit is constrained by scientific uncertainties associated with the data. The benefit of GoF information to surveillance is expected to increase over time, as the state of the science advances.
Seasonal	Vaccines	Increase the efficacy of seasonal flu vaccines by improving strain selection capabilities	Partial	9.8.5.3.1; 15.5.5.1	15.30	Near to long term	

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses							
Agent (Influenza Virus Strains)*	**Benefit Category**	**Potential Benefit**	**Unique Benefit?****	**Sections**	**Crosswalk Table(s)**	**Time Horizon for Benefit Realization**	**Time Horizon - Notes**
Seasonal, animal	Vaccines	Inform the development of universal or broad-spectrum vaccines	Partial	9.8.5.3.2; 15.5.5.2	15.31	Long term	The development of a universal or broad-spectrum vaccine represents a very scientifically challenging prospect
Evasion of Vaccines							
Seasonal, animal	Vaccines	Test whether viruses can escape protective immunity conferred by candidate universal or broad-spectrum vaccines	Yes	9.9.5; 15.3.3	N/A	Long term	The development of a universal or broad-spectrum vaccine represents a very scientifically challenging prospect
Evasion of therapeutics							
Seasonal, animal	Scientific Knowledge	Gain insight into mechanistic basis of antiviral resistance	Partial	9.10.5.1; 15.7.3	15.32; 15.33	Immediate	N/A
Seasonal, animal	Surveillance	Improve surveillance for antiviral resistance by enabling sequence-based prediction of resistance	Partial	9.10.5.2; 15.7.4	15.34	Near to long term	Information from GoF studies can be immediately applied to surveillance and downstream policy decisions, but that
Seasonal	Policy	Inform therapeutic recommendations for seasonal flu	Partial	9.10.5.3; 15.7.5.1	N/A	Near to long term	

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses

Agent (Influenza Virus Strains)*	Benefit Category	Potential Benefit	Unique Benefit?**	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Animal	Policy	Inform pandemic risk assessment of animal flu viruses and downstream decision-making about investments in pandemic preparedness activities	Partial	9.10.5.3;15.7.5.2	N/A	Near to long term	benefit is constrained by scientific uncertainties associated with the data. The benefit of GoF information to surveillance is expected to increase over time, as the state of the science advances.
Seasonal, animal	Vaccines	Improve safety of vaccine production process by identifying resistance markers that can be removed from vaccine viruses	Partial	9.10.5.4; 15.7.6	15.35	Intermediate term	FDA approval may be needed for application of GoF insights to vaccine production
Seasonal, animal	Therapeutics	Inform development of new therapeutics	Yes	9.10.5.5.1; 15.7.7.1	N/A	Long term	Development and licensing of new therapeutics is a long process
Seasonal, animal	Therapeutic	Gain insight into therapeutic's mechanism of action	Partial	9.10.5.5.2; 15.7.7.2.1	15.36	Long term	Development and licensing of new therapeutics is a long process
Seasonal, animal	Therapeutic	Facilitate regulatory approval of new therapeutics	Yes	9.10.5.5.2; 15.7.7.2.2	N/A	Long term	Development and licensing of new therapeutics is a long process

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses

Agent (Influenza Virus Strains)*	Benefit Category	Potential Benefit	Unique Benefit?**	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes
Seasonal, animal	Therapeutic	Inform development of therapeutic strategies that minimize development of resistance	Yes	9.10.5.5.3; 15.7.7.3	N/A	Long term	Development and licensing of new therapeutics is a long process
Reassortment studies							
Seasonal, animal	Scientific Knowledge	Gain insight into mechanisms driving and underlying reassortment	Partial	9.11.5.1; 15.8.3	15.37	Immediate	N/A
Seasonal, animal	Surveillance	Inform assessment of the risks posed by reassortant viruses detected through surveillance	Partial	9.11.5.2; 15.8.4	15.38	Long term	Surveillance for reassortant viruses is poor and must be improved for realization of this benefit.
Seasonal, animal	Policy	Inform pandemic risk assessment of animal flu viruses and downstream decision-making about investments in pandemic preparedness activities	Partial	9.11.5.3.2; 15.8.5.2	15.19	Long term	Surveillance for reassortant viruses is poor and must be improved for realization of this benefit.
Seasonal, animal	Policy	Inform prioritization of interventions that aim to prevent the emergence of novel reassortant viruses in human populations	Yes	9.11.5.3.1; 15.8.5.1	N/A	Near term	N/A

Table 9.2. List of Potential Benefits of GoF Research Involving Influenza Viruses

Agent (Influenza Virus Strains)*	Benefit Category	Potential Benefit	Unique Benefit?**	Sections	Crosswalk Table(s)	Time Horizon for Benefit Realization	Time Horizon - Notes

** Animal strains include avian and swine strains that have and have not infected humans. Pandemic strains include the 1918 H1N1, 1957 H2N2, and 1968 H3N2 viruses. Seasonal strains include all seasonal isolates and 2009 H1N1 pandemic isolates (now circulating seasonally). The "Agent" column includes all strain types that have been subjected to a Gain of Function using the listed approach. Of note, pandemic strains are not listed in the "Enhanced Virulence" section because while these studies include the generation of reassortant strains including genes or gene segments from pandemic strains, the resulting reassortant strains are expected to be less virulent than the wild type pandemic strains.*

***The "Unique Benefit" column indicates whether the benefit indicated in the previous column is unique or whether alt-GoF approaches can achieve the same general benefit. "No" indicates that alt-GoF approaches can provide nearly identical benefits, with respect to the quality, scope, and timeliness of the benefit; "Yes" indicates that alt-GoF approaches cannot provide the same benefit; and "Partial" indicates that alt-GoF approaches can provide similar benefits but may be limited in some way when compared to the GoF approach. Of note, a "Partial" entry does not indicate that the potential benefits of GoF and alt-GoF approaches are the same but rather that a more nuanced evaluation is needed to understand the relative benefits of GoF and alt-GoF approaches.*

**** Studies that lead to the generation of variant strains of the 1918 H1N1 pandemic virus with altered antigenicity were not identified. However, several antigenic escape studies involving a classical swine H1N1 isolate from 1930 (A/Swine/Iowa/15/30), the HA sequence of which more closely resembles the 1918 HA sequence than the sequence of any other existing isolate, were identified. Of note, this 1930 strain is not known to infect humans, although more recent classical swine viruses can infect people.*

9.2 Methodology

9.2.1 Purpose of This Task

The purpose of the qualitative benefit assessment (BA) is to provide information regarding the potential benefits of GoF research to scientific knowledge, public health, and medicine, including benefits to biosurveillance, decision-making in public health policy, and the development of vaccines, therapeutics, and diagnostics. (Throughout the report, the term "public health" is used to encompass all applied benefits to public health and medicine.) Economic benefits were not explicitly evaluated but are noted where relevant. Similarly to the risk assessment, the benefit assessment will be comparative: that is, the benefits of GoF studies are evaluated relative to the benefits of alternative experimental approaches that can provide similar information or other scientific and technical innovations that can provide similar benefits. In addition, the BA will seek to provide information regarding barriers to the realization of the benefits and the global distribution of the benefits, two key considerations when weighing the potential benefits against research risks that may be global and immediate.

9.2.2 Conceptual Approach to the Identification of Potential Benefits of GoF Research

The approach to the benefit assessment is founded on the concept that the benefits of scientific research derive from applications of new scientific information or products to unanswered scientific questions or unmet needs in public health and medicine (collectively referred to as "gaps"). To that end, a multi-step process was used to identify the benefits of GoF research relative to alternative approaches, as illustrated in Figure 9.2. First, a foundation for the analysis of benefits was established by independently (a) characterizing the expected scientific information and products derived from GoF studies of potential concern involving Pathogens with Pandemic Potential (PPPs), and (b) identifying gaps in scientific knowledge about PPPs as well as gaps in public health and medical capabilities related to the prevention and control of PPP outbreaks. Second, the scientific information/products derived from GoF research were mapped ("crosswalked") to the gaps in scientific knowledge and public health. That is, for each scientific outcome of GoF research, the gaps in scientific knowledge and public health that the information/product could address were identified; subsequently, the mechanism by which the information/production could overcome shortcomings in that gap area was determined. This crosswalk analysis was guided by the proposed benefits of GoF research, as suggested by infectious disease researchers and "translators" involved in the application of research to public health challenges. The outputs of the crosswalk analysis–GoF research applications and their downstream effects on the health of human populations–represent the potential benefits of GoF research. Third, alternate experimental approaches and/or other scientific or technical innovations that could lead to the same or similar benefits were identified. Fourth, the barriers to the realization of GoF and alt-GoF benefits were assessed, including factors that impede the translation of the research as well as "downstream" factors that limit its ultimate impact on human morbidity and mortality. Comparative analysis of the benefits afforded by GoF research versus alternative approaches, in light of the barriers to the realization of each approach, yielded insight into the unique benefits of GoF research. Fifth (not shown in Figure 9.2), the globalization potential of GoF benefits found to be uniquely beneficial were analyzed. Lastly, the impact of GoF benefits to the production of influenza vaccines on the public health burden of seasonal flu epidemics and flu pandemics was quantitatively analyzed.

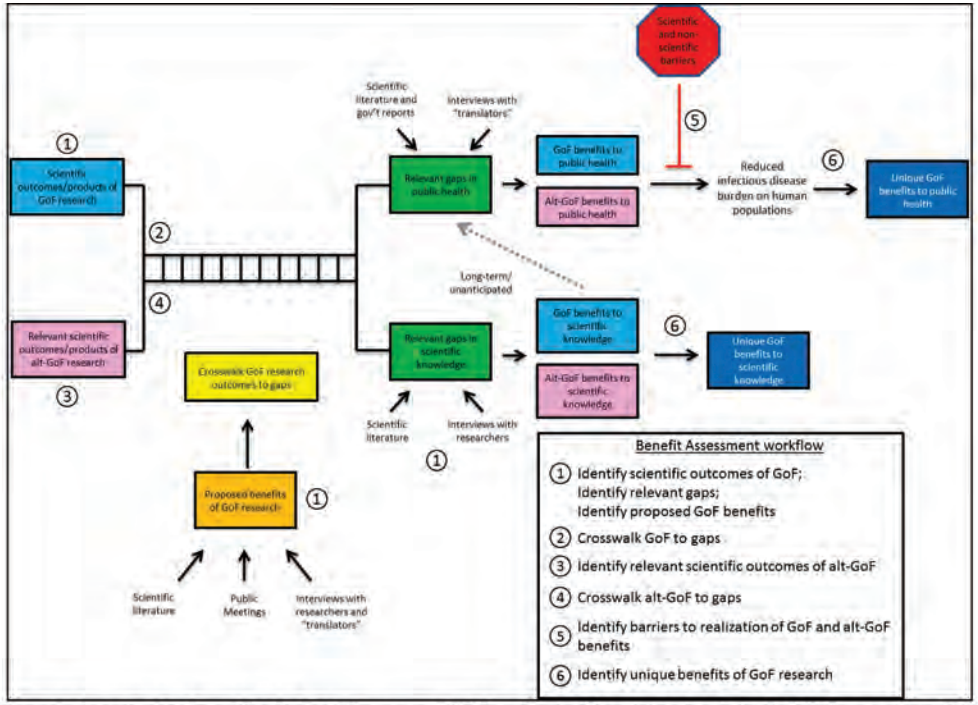

Figure 9.2. Conceptual approach and workflow for benefit assessment. "Relevant" scientific outcomes/products of alt-GoF research are those outcomes that can address similar gaps as GoF research. "Relevant" gaps in scientific knowledge and public health are those gaps that can be addressed by GoF research. Strategies for identifying the relevant outcomes and gaps will be described in detail below. Because the applications of new scientific knowledge to public health are long-term and unanticipated (e.g., whether a newly discovered virulence factor is a good therapeutic target), the barriers to realization of these long-term benefits will not be evaluated.

9.2.3 Characterizing the Expected Scientific Information and Products Derived From GoF Studies

The scientific body of work that falls within the definition of GoF research on PPPs was analyzed, as informed by the NSABB's Framework for Conducting Risk and Benefit Assessments of Gain of Function Research and the USG funding moratorium on certain types of GoF research. Specifically, this analysis included scientific research involving seasonal influenza viruses, pandemic influenza viruses (e.g., 1918 pandemic influenza virus), swine influenza viruses, and avian influenza viruses, as well as research involving SARS coronavirus, MERS coronavirus, and SARS/MERS-like bat coronaviruses. Within each field of research, all experimental approaches that are reasonably anticipated to confer one or more of the following phenotypic changes were evaluated:

- Enhanced pathogen production as a result of changes in the viral replication cycle or growth,
- Enhanced morbidity and mortality in appropriate animal models,
- Enhanced transmission in mammals, including altered host or tissue range and more efficient transmission by contact or airborne routes,
- Evasion of existing natural or induced immunity, and
- Evasion of vaccines, therapeutics or diagnostics.

Subsequently, within each GoF phenotype and for influenza viruses and coronaviruses separately, a set of general experiments that capture the range of GoF studies conducted in the published literature was defined and termed the "landscape" of GoF research. Each general experiment is described by:

- Experimental goal(s) (e.g., gain insight into mechanisms of airborne transmissibility of influenza viruses),
- Experimental approach (e.g., serial passaging of influenza virus in ferrets with selection for airborne transmission),
- Virus strains that are used (e.g., animal-origin influenza strains), and
- Expected research output(s), including new scientific information and/or products (e.g., gain insight into molecular mechanisms of airborne transmissibility of influenza viruses between mammals and identify genetic determinants of airborne transmissibility in influenza viruses).

The list of expected scientific outcomes/products of GoF research of potential concern served as the inputs of our crosswalk analysis. Specifically, scientific outcomes/products were mapped to gaps in scientific knowledge, public health, and medicine in order to assess their potential benefits to science and society.

9.2.4 Identifying Proposed Benefits of GoF Research to Scientific Knowledge, Public Health, and Medicine

Specific proposed benefits of GoF research to scientific knowledge and public health ("pro" arguments) were identified, as described by three categories of stakeholders:

- Scientific researchers who study influenza, SARS, and MERS, including those who conduct GoF studies of potential concern and those who employ alternative approaches,
- Other scientists from the public health, agricultural, and wildlife research communities, and
- "Translators" involved in applying GoF research to public health and medicine.

Critiques of proposed benefits ("con" arguments) were also identified. Proposed benefits (and associated benefit critiques) were researched in all benefit areas defined in the NSABB Framework for Conducting Risk and Benefit Assessments of Gain of Function Research (i.e., scientific knowledge, biosurveillance, medical countermeasures, decision-making in public health policy, and economic benefits). Additional benefits proposed by stakeholders that fell outside of the Framework areas were also explored.

The identification of proposed benefits and benefit critiques was carried out in several stages. First, a complete list of benefits and benefit critiques publicized by GoF stakeholders was compiled, drawing on several sources of information:

- Public meetings about GoF research, such as the October 2014 National Academies Workshop on GoF Research and past National Academies and NSABB meetings,
- Perspectives published in scientific journals, and
- Research articles and reviews published in the scientific literature.

Second, each proposed benefit and benefit critique was researched in greater detail through interviews with GoF stakeholders involved in conducting scientific research, including researchers studying influenza viruses, coronaviruses, and other infectious diseases, and stakeholders involved in translating research insights into public health practice and policy. Of note, the list of GoF stakeholders interviewed included numerous "vocal participants" in the GoF debate who had written opinion pieces about GoF research. (See "Using Interviews to Inform the BA," below, for a more detailed description of the types of stakeholders interviewed for the BA and Appendix 15.10 for a list of interviewed stakeholders.) Each interviewee was subjected to a point-counterpoint style debate about his or her proposed benefits and benefit critiques, enabling Gryphon to elucidate nuanced aspects of each argument.

Finally, this list of proposed benefits and benefit critiques was expanded upon through further examination of the scientific literature, including the basic science literature involving PPPs and the literature on infectious disease surveillance, MCM development, and public health policy. In particular, further analysis of the basic science literature was critical to identifying specific potential benefits of GoF studies to scientific knowledge.

Taken together, the information gleaned from interviews and other sources enabled the development of a list of proposed benefits of GoF research to scientific knowledge and public health and associated benefit critiques, which informed two aspects of the subsequent analyses. First, a set of public health areas that encompass all proposed GoF benefits was defined (e.g., pandemic risk assessment using surveillance data, development of influenza vaccines, etc.), which were subjected to a gap analysis as described below. Second, the list of proposed benefits of GoF research guided the crosswalk of the outputs of GoF research to gaps in scientific knowledge and public health. As described below, this crosswalk involved validation of each proposed benefit through examination and analysis of the scientific literature (for benefits to scientific knowledge) or through interviews with stakeholders in public health and MCM development

who are directly involved in applying the data or agents generated through GoF research to public health practice and policy and MCM development/production.

9.2.5 Identifying Current Practices in Medical Countermeasure Development and Production That Rely on GoF Approaches

Following the identification of the proposed benefits of GoF research, whether and how GoF approaches contribute to current practices in the development and production of influenza virus and coronavirus MCMs were explicitly determined. First, FDA regulations related to the approval of MCMs were analyzed to determine whether GoF studies facilitate or are essential for any aspects of the process, including analysis of whether resistance studies are required for the approval of new therapeutics or vaccines, the role of the Animal Rule in the demonstration of MCM safety and efficacy, and other relevant regulations. Second, the role of GoF approaches in current processes for egg- and cell-based vaccine production was reviewed through analysis of the academic literature and through interviews with industry and government personnel with expertise in influenza vaccine production. The continued application of GoF research to these areas represents one type of potential benefit of GoF research.

9.2.6 Identifying Gaps in Scientific Knowledge About PPPs and Gaps in Public Health and Medical Capabilities Related to the Prevention and Control of PPP Outbreaks

This task involved the identification of gaps in scientific knowledge about PPPs and gaps in public health and medical capabilities related to the prevention and control of PPP outbreaks that could potentially be addressed by insights gleaned from GoF research. This analysis was undertaken for several reasons. First, identification of alternative approaches that aim to address the same or similar gaps as GoF studies requires a complete and nuanced understanding of the gaps and their role in the overall public health process, as alternative approaches may benefit the same ultimate public health gap (e.g., delayed availability of vaccines during an influenza pandemic) by addressing different shortcomings in the process (e.g., increasing the rate of vaccine production versus developing pre-pandemic vaccines that can be rapidly deployed during a pandemic). Second, this gap analysis enabled identification of scientific and non-scientific barriers to the realization of the benefits.

Many gaps in public health and medicine cannot be addressed by biomedical research. Broadly speaking, the scope of this analysis was bounded by the list of GoF benefit areas defined in the task above, including biosurveillance, development and production of vaccines and therapeutics, and decision-making for public health preparedness. Within each benefit area, the list of proposed benefits was further utilized to focus on identifying and researching gaps that could be targeted by GoF research. Importantly, gaps were evaluated independently of their relationship to GoF research. To understand critical gaps in scientific knowledge about PPPs, the state of the science regarding how influenza viruses, SARS-CoV, and MERS-CoV are transmitted between hosts, cause disease, overcome protective immunity, and evolve new phenotypic characteristics was reviewed. Interviews with researchers and "translators," as well as an analysis of the scientific literature, provided information about gaps in public health and medicine. Notably, this research attempted to identify not only the gaps that could be addressed by GoF studies, but also who may use the outputs of GoF studies to address the gaps, so that these stakeholders could be interviewed to validate the assessment of the benefits of GoF research (described below).

9.2.7 Crosswalking GoF Research Outcomes to the Gaps in Scientific Knowledge, Public Health, and Medicine

The next phase of the analysis determined how the research outputs of GoF studies can address gaps in scientific knowledge, public health, and medicine. This "crosswalk" was guided by the proposed benefits of GoF research. Each proposed benefit was validated through analysis of the scientific literature (for benefits to scientific knowledge) or through interviews with "translators" who are directly involved in applying the data or agents generated through GoF studies to public health and MCM development/production. Critically, this analysis included an assessment of the relevance and validity of all benefit critiques previously identified, including concerns about whether and when the benefits will be realized. Throughout the benefit validation process, GoF stakeholders were re-engaged as needed to solicit additional information necessary to validate a given benefit or benefit critique or to clarify previous remarks.

Benefits to scientific knowledge have intrinsic value while benefits to public health apply to "upstream" aspects of the public health process (e.g., biosurveillance), the ultimate goals of which are reducing human morbidity and mortality caused by influenza viruses and coronaviruses. To understand all of the steps needed to realize the public health benefits from discovery to immediate application to ultimate impact on public health, the analyses of public health systems were leveraged. For example, genetic markers that confer high growth to vaccine viruses, identified through GoF studies, are incorporated into vaccine viruses used for manufacturing in order to shorten production timelines by increasing the rate of viral antigen production. In turn, these improvements to the vaccine production process lead to faster vaccine availability during a pandemic, which reduces morbidity and saves lives. Finally, for benefits related to the production of influenza vaccines, the effects of improving the availability vaccines on human morbidity and mortality during outbreaks were further evaluated using quantitative methods, as described in the "Quantitative Analysis of GoF Benefits" section below. Collectively, the outputs of the crosswalk analysis – GoF research applications and their downstream impacts on the health of human populations – represent the potential benefits of GoF research. Notably, realization of some public health benefits may depend on other scientific and non-scientific factors, the implications of which are explored in our assessment of barriers to the benefits, described below.

9.2.8 Assessing the Barriers to the Realization of GoF and Alt-GoF Studies

One of the most challenging aspects of weighing the risks and benefits of GoF research is that there is a temporal mismatch between the risks and the benefits of the research–the risks are assumed at the time the research is conducted, while the benefits to public health and medicine *may* accrue in the future. To enable the comparison of risks and benefits, the benefit assessment is structured to provide data about the probability and likelihood that the potential benefits of GoF research will be realized.

To accomplish this goal, benefits to scientific knowledge and benefits to public health/medicine were considered separately. Scientific insights have immediate intrinsic value and may also inform the development of novel vaccines or therapeutics, surveillance strategies, and other advancements in public health/medicine in the future. Because the nature and timing of such applications are difficult to predict with certainty, this report acknowledges but does not attempt to elucidate or evaluate the unforeseen applications of basic science research to public health or medicine for this analysis.

In contrast, the potential benefits of GoF research to public health/medicine involve clear applications of scientific information gleaned through GoF studies to unmet needs in public health. However, unlike the risks, which pose possible direct threats to humans, animals, and the environment, the benefits involve "upstream" aspects of the public health process, and evaluating how and when the benefits will improve

the health of human populations is complex. That is, translation of the research may depend on other scientific, technical, and regulatory factors (e.g., the need to gain FDA approval in order to market a new therapeutic). Additionally, gaps or inefficiencies in downstream aspects of the public health process (e.g., limited funding for investment in the development of pre-pandemic vaccines) may limit the ultimate impact of the research application on human health. Collectively, these factors function as "barriers" that reduce the likelihood and delay the timing of the realization of the benefits, although significant uncertainties in when and whether barriers can be overcome preclude a meaningful quantitative estimate of either parameter.

For those validated benefits to public health, the barriers that may impede or delay realization of the benefits were identified in two stages. First, the state of the science and the limitations of the experimental approach that could influence the nature and scope of the benefit were considered. For example, a set of mutations that confer efficient transmissibility to one strain of zoonotic influenza, identified through a GoF experiment, may not lead to the same phenotypic changes in a different genetic context, and the current ability to predict the phenotypic consequences of mutations in new strains is sub-par. Together, these sources of scientific uncertainty represent scientific barriers that compromise the utility of this information in aiding analysis of biosurveillance data. Subsequently, scientific advancements needed to overcome these scientific uncertainties were defined.

Second, the gap analysis of public health capabilities was leveraged to elucidate the non-scientific barriers to the realization of each potential benefit and to determine the type of resources or advancements that are required to overcome or circumvent each barrier. These advancements include investments in public health infrastructure (e.g., expanding global influenza surveillance networks), investments in MCM development infrastructure (e.g., increasing the number of cell-based and other non-traditional influenza vaccine production facilities), regulatory approval of new MCMs or MCM production processes, and changes in public health policies or regulations. This analysis was informed by the concerns related to benefit realization that were identified in the literature and through interviews with stakeholders. For all aspects of this task, scientists, public health practitioners, MCM developers, public health policy-makers, and other GoF stakeholders previously interviewed were re-engaged as needed to clarify opinions regarding GoF benefits and benefit critiques, challenges in biosurveillance, MCM development, public health policy-making, and other topics.

9.2.9 Assessing if Alternate Experimental and Other Scientific Innovations Could Lead to the Same Benefits

GoF studies comprise a subset of all research activities involving PPPs, and some alternative approaches may pose less risk than GoF studies but yield the same or similar benefits. Two types of "alt-GoF" approaches were considered. First, alternative experimental approaches that can address the same scientific questions as GoF approaches were identified, for example Loss of Function versus Gain of Function approaches for identifying determinants of pathogenicity. The second type of alt-GoF approach considered is other scientific and technical approaches that can address the same public health gaps that GoF can address, but using a completely different strategy. For example, GoF studies that increase the yields of influenza vaccine viruses in eggs or cell culture may benefit influenza vaccine production by shortening the time needed to produce the same number of vaccine doses. However, a completely different strategy, such as the production of recombinant influenza vaccines using insect cells, may also address issues related to the timeliness and amount of vaccine available even though this alternate approach shares no experimental features with the GoF approach. After considering these alt-GoF approaches, the benefit assessment can identify those types of GoF studies that may provide unique benefits to scientific knowledge and public health, which will complement the analysis of the net risks associated with the conduct of GoF research relative to alternative approaches.

Alternative approaches that may yield similar information or public health impacts as GoF research were identified by drawing on the alternative approaches suggested by infectious disease researchers and translators during public meetings about GoF research (such as NSABB meetings and NAS symposia), in perspectives published in scientific journals, and during interviews, as well as the scientific literature on PPPs. Importantly, alt-GoF research spans a wide range of topics, and those alt-GoF studies that yield information outside the scope of GoF research are not relevant for the analysis. For this reason, to focus the analysis on those approaches that may inform the same or similar gaps as GoF research, alt-GoF approaches were identified by starting with the set of scientific knowledge gaps that are targeted by GoF studies and referencing the scientific literature to identify alt-GoF approaches that target those same gaps. The analysis of alternative approaches that target similar public health gaps critically leveraged the analysis of public health systems, in particular the understanding of how the steps from discovery to application of GoF research participate in an overall system.

Subsequently, the potential benefits of alt-GoF approaches were identified through the same process as for GoF studies: a crosswalk of the research outputs of alt-GoF studies or the products of alternative scientific/technical innovations to gaps in scientific knowledge and public health that can be addressed by GoF research. Similarly, the barriers to the realization of alt-GoF benefits were assessed through identification of co-factors needed for the translation and downstream public health impacts of alt-GoF approaches.

Ultimately, the goal of the benefit assessment is to identify the benefits of GoF research of concern relative to alternative experimental approaches that may pose less risk. A list of benefits was compiled, as well as the scientific and non-scientific co-factors required for realization of each benefit, for each GoF research approach of potential concern. To provide a comparison, a similar list was compiled for each alt-GoF approach evaluated. Evaluation of the unique benefits involved comparison of GoF and alt-GoF benefits, in light of barriers to realization of each set of benefits. To identify the unique benefits of GoF research to scientific knowledge, the benefits of GoF research and those of alternative experimental approaches were compared. Identification of the unique benefits of GoF research to public health involved additional comparison of the benefits of GoF research to those of alternative scientific and technical innovations that address the same public health gap through different mechanisms. Beyond an explicit consideration of barriers, a variety of factors were considered when comparing the benefits of GoF research to alt-GoF research, including the ability of an approach to:

- Provide causative versus correlative (associative) data,
- Provide direct evidence of a phenomenon versus indirect evidence (e.g., showing that pathology changes by manipulating the virus vs manipulating the host of a virus),
- Provide the ability to predict potential natural phenomena in the future versus describe the current state of nature,
- Provide evidence in the near term versus the far term, and
- Provide needed evidence with the least effort and resources, including financial resources and laboratory animals (efficiency).

9.2.10 Evaluating the Globalization Potential of GoF Benefits

Whether risks and benefits are equally distributed across populations is also an important consideration in any risk-benefit comparison. For GoF research involving PPPs, the risks– that biosafety or biosecurity incidents associated with the conduct of GoF research involving PPPs may spark a pandemic–are global. In contrast, whether GoF benefits are globally distributed is likely to vary by the type of benefit considered. The extent to which these benefits can be globalized influences whether risks and benefits are equally distributed for a particular type of GoF study. To inform NSABB's deliberations on this issue, the benefit assessment qualitatively assessed the globalization potential of the latter set of GoF benefits, through analysis of historical case studies examining the globalization of similar benefits and through review of relevant USG policies (e.g., policies related to MCM sharing, etc.).

The globalization potential of select GoF benefits, namely those that are relevant worldwide but may be primarily realized in the US and other developed countries, were evaluated. To support this task, USG policies, programs, and international agreements relevant to globalization of GoF benefits were analyzed, including USG policies and international agreements regarding MCM sharing during global outbreaks and other relevant pandemic preparedness support for the World Health Organization (WHO). Also, historical examples of USG involvement in the globalization of GoF benefits were analyzed, considering the context of the historical example and its relevance to a future outbreak of influenza, SARS, or MERS. Taken together, these analyses will enable qualitative assessment of the degree to which the USG promotes globalization of various GoF benefits, as well as the timescale over which those benefits are expected to internationalize.

9.2.11 Quantitative Analysis of GoF Benefits

Although the ability to provide quantitative metrics for benefits would facilitate comparison of the benefits of GoF versus alt-GoF research as well as of the risks and benefits associated with particular types of GoF studies, given the differences in the availability and quality of data related to the realization of the benefits, a quantitative analysis of all benefits cannot be performed. In particular, benefits related to some aspects of MCM development, surveillance, public health policy, and scientific knowledge are associated with multiple sources of uncertainty in how, when, and where the benefits will ultimately improve the health of human populations, which precludes a meaningful quantitative analysis of the magnitude of those effects. However, it is hoped that the rigorous examination of the pathways through which those benefits lead to reductions in the burden of infectious diseases on human populations provide a qualitative sense of the potential scale of each benefit, in light of current barriers to the realization of that benefit.

Benefits related to the production of influenza vaccines are amenable to quantitative analysis, which leverages models developed for the biosafety RA (specifically the nested SEIR models of global outbreaks) to parametrically explore how changes in the control of outbreaks of PPP can mitigate morbidity or mortality. Critically, many factors prevent the absolute assignment of a particular GoF outcome to a quantitative benefit. For this reason, the quantitative approach herein shows how changing a public health or medical capability that can be targeted by GoF research (such as the timeliness of the availability of a vaccine during a pandemic) could affect the consequences of a global outbreak. These data are accompanied by a commentary on the barriers for GoF achieving a desirable change to public health and medical capabilities or preventing a deterioration of public health/medical capabilities so that stakeholders can understand the probability of achieving the quantitative benefits modeled. This quantitative component of the evaluation was accomplished using the HHS-BARDA Interactive Influenza Model (as described in the biosafety RA described above) to parametrically analyze the effect of:

1. The timeliness of availability of a vaccine after an seasonal or pandemic influenza outbreak, and
2. The amount of vaccine available when it becomes available.

For each of these parameters, the value of the parameter was allowed to vary from arbitrarily large numbers to arbitrarily small numbers during simulations of outbreaks of seasonal influenza and pandemic influenza (similar to the pandemic strain of 1918 or 2009). This enabled determination of the value at which each of these parameters begin to affect the consequence of global influenza outbreaks. The change in parameter value needed to significantly change the consequence of a global outbreak was compared with the plausible benefit to the vaccine afforded by GoF and alt-GoF studies, in order to determine if either is likely to have the significant effect on the consequences of an outbreak. Moreover, for GoF studies that are necessary to maintain the status quo for influenza vaccines, this analysis determined how much worse an outbreak would be if those studies were not allowed to continue.

9.2.12 Using Interviews to Inform the Benefit Assessment

As described above, interviews with GoF stakeholders critically informed many aspects of the BA, namely:

- Identification of proposed benefits of GoF research to scientific knowledge and public health, as well as associated benefit critiques,
- Identification of alt-GoF approaches that may yield the same or similar as GoF approaches,
- Validation of the proposed benefits of GoF and alt-GoF research, in particular validation of benefits to public health, and
- Identification of scientific and non-scientific barriers that may impede the realization of GoF and alt-GoF benefits.

To inform each of these steps, Gryphon Scientific reached out to 78 stakeholders from a variety of sectors for interviews, 52 of who agreed to participate in an interview or site visit, resulting in an overall response rate of 66%. The breakdown of response rates by sector is as follows: ~50% for government stakeholders, 80% for industry stakeholders, and ~70% each for non-PPP researchers and PPP researchers. Gryphon staff visited seven influenza and coronavirus research laboratories to collect additional data for the risk assessment through laboratory tours and interviews about biosafety and biosecurity practices (Appendix 15.10). During the site visits, Gryphon also questioned principle investigators and their senior research staff, postdoctoral fellows, and senior graduate students about the benefits of their research to scientific knowledge and public health. These additional discussions with senior researchers and trainees boosted the total number of PPP researchers interviewed for the project.
For interviews focused exclusively on the benefits of GoF research, local interviews were carried out in person, while all other interviews were conducted over the phone. In total, 86 stakeholders were interviewed (Figure 9.3).

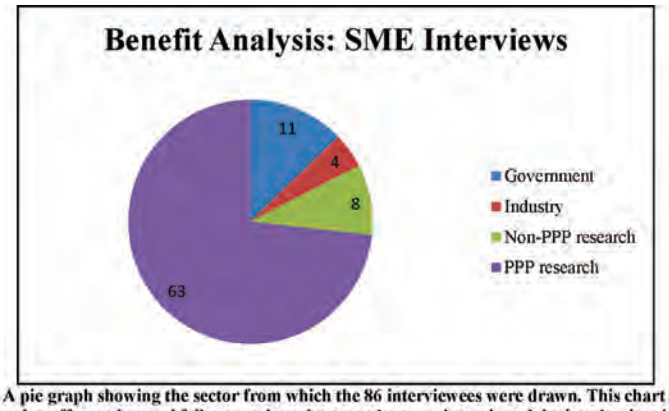

Figure 9.3. A pie graph showing the sector from which the 86 interviewees were drawn. This chart includes senior research staff, postdoctoral fellows, and graduate students we interviewed during the site visits to labs that conduct PPP research. "Translators" include government and industry personnel, as well as some PPP researchers who are involved in translation activities, such as WHO strain selection meetings for the seasonal influenza vaccine. Of note, several government personnel are also actively involved in PPP research.

Given the important role of interview data in the BA, several points concerning the breakdown of interviewees by sector bear further discussion. First, stakeholders from multiple sectors are involved in the conduct and application of GoF research. Specifically, in addition to PPP researchers, several government personnel (e.g., CDC personnel) are actively involved in PPP research. Industry stakeholders may also conduct GoF research, in particular research that enhances the production of influenza viruses in the influenza vaccine production industry. Conversely, regarding "translation" of the benefits to public health/medicine, in addition to government and industry personnel, several PPP researchers participate in translation activities. In particular, PPP researchers are involved in the application of GoF research to biosurveillance, including conducting pandemic risk assessments and participating in WHO strain selection meetings for seasonal influenza vaccines. Second, a diversity of opinions was expressed by stakeholders within all sectors. That is, within each sector, interviewees both espoused and critiqued potential benefits of GoF research. Put another way, multiple "con" arguments were made by those who conduct PPP research, and multiple "pro" arguments were suggested by non-PPP researchers, as well as the converse.

In this context, one salient point is that a greater number of PPP researchers were interviewed than non-PPP researchers. Although the BA would be further strengthened through additional input from stakeholders in every sector, in particular non-PPP researchers and industry stakeholders, the number of the interviews conducted was necessarily limited by the compressed timescale of the project. Gryphon's strategy for selecting the set of interviewees was to ensure that the interviews spanned all unique arguments pertaining to GoF research benefits and benefit critiques. The interviewee list evolved over time, in response to the information and suggestions provided by prior interviewees. Notably, PPP researchers, given their deep and broad expertise in the fields of influenza and coronavirus research, were generally able to speak with much greater depth and nuance about the scientific benefits and caveats associated with both GoF and alt-GoF approaches than non-PPP researchers. As a result, the list of benefits discussed during interviews with non-PPP researchers became "saturated" – that is, additional interviews did not yield novel insights about potential GoF benefits – more quickly than those discussed during interviews with PPP researchers. This phenomenon was one reason that a greater number of interviews with PPP researchers were conducted. A second reason stems from the fact that interviews

with translators informed the validation of proposed benefits. These interviews necessarily targeted those who are directly involved in the applications of GoF research, which included numerous PPP researchers (but not non-PPP researchers).

A second salient point is that the suite of PPP researchers interviewed includes researchers who use GoF approaches, as well as researchers who primarily use alt-GoF approaches but who collaborate with GoF researchers and are co-authors on papers containing GoF experiments. Strikingly, none of the PPP researchers who exclusively publish papers involving alt-GoF approaches were willing to participate in interviews. (One declined and four did not respond to Gryphon's invitation.) Of note, given the broad definition of GoF research provided in the NSABB Framework and used in this assessment, nearly all PPP researchers who engage in "wet lab" research utilize GoF approaches, complicating the identification of a large cohort of PPP researchers who exclusively conduct alt-GoF approaches. Alt-GoF researchers who were contacted primarily employ computational, sequence-based (i.e., phylogenetic analysis), or *in vitro*, virus-free approaches (e.g., biochemical approaches, structural biology approaches, etc.). Importantly, all GoF researchers also use alt-GoF approaches, for a variety of reasons, including risk mitigation, to complement information gleaned from GoF approaches (e.g., GoF and LoF experiments), or when an alt-GoF approach can more effectively answer a particular scientific question than a GoF approach. Collectively, the set of PPP researchers who were interviewed have direct experience conducting nearly every alt-GoF approach identified in this assessment and thus could speak with authority on the scientific knowledge benefits of both GoF and alt-GoF approaches. Because PPP researchers who exclusively employ alt-GoF approaches declined to be interviewed, the question of whether they have substantively different viewpoints on the benefits of alt-GoF approaches could not be determined.

9.3 Coronaviruses: Benefits of GoF research

9.3.1 Summary

This section describes the benefits of GoF research involving coronaviruses (CoVs), which includes (1) approaches that enhance virus production, (2) alter host range, (3) enhance virulence in appropriate animal models, and (4) lead to evasion of therapeutics. Such GoF studies were found to generate scientific knowledge, have direct applications to the development of vaccines and therapeutics, and may also have economic benefits (not considered). Alt-GoF approaches that may generate similar benefits were also identified and analyzed. At present, GoF studies involving CoVs have unique and direct benefits, particularly to the development of vaccines and therapeutics.

9.3.1.1 GoF Approaches That Enhance Virus Production

9.3.1.1.1 Benefits to Scientific Knowledge

- GoF approaches that enhance virus production have potential to enable the development of in vitro model systems for the study of any animal CoV in a variety of cell types, including immortalized and primary cell lines. However, the fact that few animal CoVs identified to date can be grown in existing cell culture systems limits the success of this approach.

9.3.1.2 GoF Approaches That Alter Host Range

9.3.1.2.1 Benefits to Scientific Knowledge

- GoF approaches:

- Are uniquely capable of identifying novel viral genetic traits and factors that contribute to cross-species adaptation, in any CoV strain.
- Are uniquely capable of demonstrating that a particular mutation(s) is necessary and sufficient to alter the host range of a coronavirus.
- Enable the development of in vitro model systems for the study of any animal CoV in a variety of cell types, including immortalized and primary cell lines, and
- Uniquely enable the development of animal models that recapitulate human disease pathogenesis, which can be used to study many facets of disease pathogenesis, including the role of viral and host immune factors in host pathology and the role of tissue tropism in pathology.

- Alternative approaches:
  - Comparative sequence analysis is uniquely capable of identifying genetic traits that are associated with human adaptation, but this approach is limited to the study of CoVs that have already caused human infections and is significantly constrained by the quality and availability of genetic surveillance data for CoVs. In addition, the causality of mutations must be confirmed through a GoF experiment.
  - In vitro approaches, including characterization of the capacity of wild type viruses to infect cells derived from various host species, the use of other viruses pseudotyped with CoV Spike proteins, and binding assays using recombinant proteins, are limited to studying the role of the Spike protein in cross-species adaptation. In addition, results using pseudotyped viruses or recombinant proteins may not be recapitulated in the context of the wild type virus.
  - Use of naturally permissive cell lines to study bat CoVs is limited to the few bat CoVs that can productively infect and replicate within existing cell culture lines.
  - Use of cell lines ectopically expressing permissive receptor proteins to study bat CoVs is limited to cell lines that can be readily transfected, and modifications to cell lines may alter the biology of infection.
  - Naturally susceptible hosts of SARS and MERS cannot be used to study disease pathology because they are asymptomatic or display different symptoms from humans.
  - Transgenic animals that are expressing human receptor proteins do not recapitulate human disease pathogenesis, thus results using transgenic animals may not translate to humans.
  - Though human autopsy data provides direct information about human pathology, limited autopsy data are available and mortalities are not representative of all cases, limiting the generalizability of results.
  - Alternative coronaviruses such as mouse hepatitis virus (MHV) can be used to gain insight into basic aspects of CoV biology but are sufficiently distinct from human CoVs that they are not suitable for the study of pathogenesis.

9.3.1.2.2 Benefits to Vaccine Development

- GoF approaches:

- Uniquely enable the development of animal models that recapitulate human disease pathogenesis, which support testing of the safety and efficacy of candidate vaccines in a robust system that can be used to demonstrate that vaccines reduce disease-associated pathology and can reveal whether vaccines have adverse side effects, and
- Are uniquely capable of providing reliable information about the broad-spectrum potential of CoV vaccines, through the use of chimeric bat-SARS CoVs as vaccine challenge viruses.

- Alt-GoF approaches:
  - Other animal models (naturally susceptible hosts and transgenic animals) do not recapitulate human disease pathogenesis, and thus are weak systems for demonstrating the efficacy of vaccine candidates and cannot reveal adverse side effects.
  - Few wild type bat CoVs can be cultured in existing cell lines, and bat CoVs do not naturally infect mice, thus wild type bat CoVs have limited utility for the development of broad-spectrum vaccines.
  - Vaccine efficacy results using viruses pseudotyped with CoV Spike proteins must be confirmed in wild type (or chimeric CoV strains) due to significant differences in the surface presentation of Spike proteins.

9.3.1.2.3 Benefits to Therapeutic Development

- GoF approaches:
  - Uniquely enable the development of animal models that recapitulate human disease pathogenesis, which support testing of the safety and efficacy of candidate therapeutics in a robust system that can be used to demonstrate that therapeutics reduce disease-associated pathology,
  - Are uniquely capable of providing reliable information about the broad-spectrum potential of CoV therapeutics, through the use of chimeric bat-SARS CoVs as vaccine challenge viruses.

- Alt-GoF approaches:
  - Other animal models (naturally susceptible hosts and transgenic animals) do not recapitulate human disease pathogenesis, and thus are weak systems for demonstrating the efficacy of vaccine candidates and do not satisfy the FDA Animal Efficacy Rule.

9.3.1.3 GoF Approaches That Enhance Fitness or Virulence in Cell Culture or Animal Model Systems

It should be noted that serial passaging of viruses in mice both alters the host range of the virus and enhances its virulence in mice. The value of GoF benefits derived from the use of mouse-adapted viruses, relative to alternative approaches, was summarized in Section 9.3.1.2 (GoF approaches that alter host range) and will not be repeated in this section.

9.3.1.3.1 Benefits to Scientific Knowledge

- GoF approaches:
  - Represent the most efficient and effective strategy for identifying novel genetic traits and viral factors that contribute to virulence, in any CoV strain, and

- Are uniquely capable of demonstrating that a particular mutation(s) is necessary and sufficient to enhance the fitness/virulence of a coronavirus.

- Alt-GoF approaches:
  - Comparative sequence analysis is uniquely capable of identifying genetic traits that are associated with enhanced virulence in humans but is limited to the study of SARS and MERS and is significantly constrained by the quality and availability of genetic surveillance data for CoVs. In addition, any hypotheses must be experimentally confirmed.

  - Loss of Function approaches (i.e., screening gene knockout viruses in vitro) are limited to the discovery of viral factors involved in replication and may uncover factors that indirectly contribute to virulence. Though targeted mutagenesis can be used to confirm that a genetic trait is necessary for virulence, this LoF approach provides limited information about how proteins cooperate to enhance virulence, which is a complex, multi-genic trait.

9.3.1.3.2 Benefits to Vaccine Development

- GoF approaches:
  - Are uniquely capable of determining whether live attenuated vaccine viruses (LAVs) recover virulence upon growth in cells or animals, a critical aspect of safety testing for this type of vaccine, and

  - Represent the most efficient and effective strategy for identifying novel virulence factors, which can be deliberately attenuated to generate LAVs, a promising type of CoV vaccine platform.

- Alt-GoF approaches:
  - Alternative experimental approaches for identifying virulence determinants are less efficient than GoF approaches and are primarily limited to the study of known virulence factors, limiting their utility for informing LAV development.

  - Other types of vaccine platforms that do not rely on GoF approaches have strengths and limitations relative to LAVs, which may rely on GoF for their development.

9.3.1.3.3 Benefits to Therapeutic Development

- GoF approaches:
  - Represent the most efficient and effective strategy for identifying novel virulence factors, which are potential therapeutic targets.

- Alt-GoF approaches:
  - Alternative experimental approaches for identifying virulence determinants are less efficient that GoF approaches and are primarily limited to the study of known virulence factors, limiting their utility for discovering potential new therapeutic targets.

  - High-throughput screening of small molecule compounds for their ability to reduce viral replication in vitro has generated promising therapeutic candidates, but such screens are limited to the discovery of drugs that inhibit viral replication, only one aspect of virulence.

- High-throughput screening of monoclonal antibodies (mAbs) for their ability to bind CoV Spike proteins has generated promising therapeutic candidates, but mAb-based therapeutics have several drawbacks, including the fact that CoV Spike proteins can readily acquire escape mutations.

9.3.1.4 GoF Approaches That Lead to Evasion of Therapeutics in Development

9.3.1.4.1 Benefits to Therapeutic Development

- GoF approaches:
  - Are uniquely capable of determining the genetic threshold for resistance of a candidate therapeutic prior to field deployment of the therapeutic, which is a recommended component of an Investigational New Drug application to the FDA,
  - Are uniquely capable of identifying the viral target of a novel therapeutic with an unknown mechanism of action,
  - Provide insight into the mechanism of activity of a therapeutic through the identification of mutations that are necessary and sufficient to confer resistance to the therapeutic, which is a recommended component of an Investigational New Drug application to the FDA, and
  - Are uniquely capable of determining the therapeutic dose that is least likely to lead to the acquisition of antiviral resistance as well as determining whether combination therapies better prevent the emergence of resistant viruses than individual therapies, which informs the development of therapeutic strategies that will be effective for a longer time in the field.
- Alt-GoF approaches:
  - X-ray crystallography and photoaffinity crosslinking are limited to the study of therapeutics with known viral targets, and inferring mechanistic information based on static data about drug-viral interactions may be difficult.
  - RNAi screens to identify host factors that are required for the antiviral activity of a therapeutic provide indirect information about the mechanisms of therapeutics that target viral proteins.

GoF approaches that benefit the development of vaccines and therapeutics may lead to downstream economic benefits, which were not analyzed in this report. GoF approaches involving coronaviruses do not benefit surveillance, informing policy decisions, or the development of diagnostics.

9.3.2 Overview of the GoF Research Landscape Involving Coronaviruses

This assessment describes the benefits of GoF experiments involving SARS-CoV, MERS-CoV, and SARS/MERS-like bat CoVs. From a review of the coronavirus literature, experimental approaches were identified that are reasonably anticipated to lead to the following phenotypic changes:

- Enhanced pathogen production as a result of changes in the replication cycle or growth,
- Altered host range (typically accompanied by enhanced virulence in the new host),
- Enhanced fitness or virulence in cell culture or laboratory animal model systems respectively, and

- Evasion of therapeutics in development.

As current animal models for studying coronaviruses do not support transmission between animals, this field does not include any approaches that lead to enhanced transmission in appropriate animal models. Additionally, because there is no widespread population immunity to the coronaviruses and there are no licensed coronavirus vaccines, this field does not include any approaches that lead to evasion of existing natural or induced immunity. Finally, no coronavirus research that is reasonably anticipated to lead to evasion of diagnostics or of vaccines in development was identified. (Additionally, there are currently no FDA-approved vaccines or therapeutics for coronaviruses.)
Of note, the four human coronaviruses that cause mild to moderate respiratory illnesses such as the common cold or croup (coronaviruses HKU1, OC43, 229E, and NL63) were not evaluated because these are not considered in the NSABB GoF Framework. Throughout this report, the use of the term "coronaviruses" or "CoVs" refers specifically to SARS-CoV, MERS-CoV, and SARS/MERS-like bat CoVs such as HKU4 and HKU5.

The following chapter summarizes the results of the assessment of the benefits of GoF research involving coronaviruses. A more detailed analysis to further support the findings described in Chapter 9.3 is presented in Appendix IV Section 15.I. As the relative ability of a given GoF (or alt-GoF) approach to address a particular scientific knowledge or public health gap often hinges on nuanced differences between the benefits and limitations of different approaches, readers who seek an in-depth understanding of the benefits of GoF research are directed to chapter 15.

In the following section, a brief overview of the experimental approaches within each GoF phenotypic category is provided and the scientific outcomes and/or products of each approach are described.

9.3.2.1 Experimental Approaches That Lead to Enhanced Pathogen Production

Serial passaging of CoV in cell culture leads to the generation of higher-yield viruses. This approach is used to enhance the growth of viruses with naturally poor growth properties, in order to develop an *in vitro* model system for experimental use.

9.3.2.2 Experimental Approaches That Alter host Tropism in Mammals

Several experimental approaches alter the host range of CoVs. One approach involves "Spike swapping" – that is, targeted genetic modification to replace all or part of the coronavirus Spike protein, a viral surface protein that mediates virus entry into cells and is a critical determinant of host restriction, with the Spike protein from another CoV species. This manipulation leads to the generation of a recombinant, chimeric CoV that may exhibit altered host tropism relative to the parental CoV species. The purpose of these experiments is three-fold:

- Introducing the SARS Spike protein into the backbone of bat CoVs, which do not efficiently infect standard cell culture lines or animals, enables the chimeric virus to infect cells/animals, thus creating a tool that can be used to study the biology of the bat CoV,

- Chimeric viruses are used as tools to test whether CoV therapeutics and vaccines are broad-spectrum, capable of protecting against potentially emerging SARS/MERS-like bat CoVs as well as SARS and MERS, and

- Testing the ability of chimeric CoVs to infect various types of cells and animals reveals the breadth of host tropism conferred by a given Spike protein, and comparing the sequences of

parental and donated Spike proteins with different host tropism can uncover amino acid residues that mediate host restriction.

A second approach that leads to altered host range involves serial passaging of CoVs in mice, which leads to the generation of viruses that have adapted to more efficiently infect and cause disease in mice. The purpose of this experiment is two-fold:

- Mouse-adapted strains are experimental tools that are used for the study of disease pathogenesis and for testing the efficacy and safety of vaccines and therapeutics, and
- Comparing the sequences of the mouse-adapted and the parental strain leads to the identification of mutations that are associated with adaptation, which provides a foundation for follow-up studies investigating the mechanistic basis of virus adaptation to new hosts.

A final approach involves targeted mutagenesis to introduce mutations that are associated with altered host tropism, which is performed to demonstrate that the mutation(s) are necessary and sufficient to alter host tropism. This information provides a foundation for follow-up studies investigating the phenotypic traits underlying virus adaptation to new hosts.

9.3.2.3 Experimental Approaches That Enhance Fitness or Virulence in Cell Culture or Laboratory Animal Model Systems

Several experimental approaches enhance the fitness or virulence of CoVs in cell culture or laboratory animal model systems, respectively. First, serial passaging of CoVs in mice leads to the generation of viruses with both enhanced infectivity to and virulence in mice. Because of the specificity of virus-host interactions that are important determinants of host tropism and pathogenicity, this adaptation often translates to reduced virulence in humans. The purpose of this experiment is two-fold:

- Enhancing the virulence of the virus in mice is an important aspect of creating a mouse model that replicates human disease pathology, which is needed for the study of disease pathogenesis mechanisms and the testing of medical countermeasures, and
- Comparing the sequences of the mouse-adapted and the parental strain leads to the identification of mutations that are associated with enhanced virulence, which provides a foundation for follow-up studies to elucidate the mechanistic basis of virulence. This information can also benefit public health by identifying new potential targets for therapeutics or for attenuation, in order to create attenuated vaccine viruses.

A second approach involves targeted genetic modification of viruses to introduce mutations that are associated with enhanced virulence, which is performed to demonstrate that the mutation(s) are necessary and sufficient to enhance virulence. This information provides a foundation for follow-up studies to elucidate the mechanistic basis of virulence.

A third approach involves serial passaging of attenuated viruses that are candidate live attenuated vaccines, in order to determine whether the viruses acquire mutations that enhance fitness/virulence. Because LAVs with an ability to recover fitness during growth *in vivo* could cause adverse outcomes in people, a negative result is an important indicator of safety for any live attenuated vaccine in development.

9.3.2.4 Experimental Approaches That Lead to Evasion of Therapeutics in Development

Serial passaging of a virus in cells in the presence of a therapeutic may lead to the emergence of viruses that are resistant to inhibition/neutralization by that therapeutic. The purpose of the experiment is to understand whether and how readily resistance will arise in response to selective pressure from the therapeutic and to identify mutations that are associated with resistance to the therapeutic, which provides a foundation for follow-up studies investigating the mechanisms underlying antiviral activity and antiviral resistance. This information benefits the development of these therapeutics. Specifically, emergence-of-resistance data speaks to the potential field efficacy of the therapeutic, and information on both antiviral mechanism and emergence of resistance are important components of an investigational new drug application to the FDA.

9.3.3 Identification of Potential Benefits and Limitations of GoF Research Involving CoVs

In this section, the potential benefits of GoF research involving CoVs in each benefit category listed in the NSABB Framework are evaluated.

9.3.3.1 Benefits and Limitations of GoF to Scientific Knowledge

9.3.3.1.1 Scientific Knowledge Benefit 1: Gain Insight into the Mechanisms Underlying Adaptation of Animal CoVs to Humans

SARS and MERS unexpectedly emerged from their animal reservoirs to infect humans in 2002 and 2012, respectively. Surveillance of bats and other CoV reservoir species indicates that there is a large diversity of animal CoVs circulating in nature, including many species that are genetically related to SARS and MERS and thus may have the potential to spill over into human populations in the future.[483,484,485,486] Although multiple coronaviruses have been shown to exhibit a flexible capacity for cross-species transmission,[487,488] the mechanisms underlying CoV adaptation to new host species are poorly understood.

Several GoF approaches have potential to address this scientific knowledge gap. Serial passaging of CoVs in cells derived from a non-natural host organism or in a non-natural laboratory animal host selects for viruses that more efficiently infect cells/animals, thereby enabling the identification of mutations that are sufficient for adaptation to a new host species. Identifying where mutations arise during adaptation to new hosts points to viral factors that may play a role in adaptation, and studying the phenotypic consequences of the mutations provides insight into the mechanistic basis of cross-species adaptation. One key benefit of this approach is that it can lead to the discovery of novel genetic traits and virus proteins that are involved in the process of adapting to new hosts without the need for prior knowledge of viral adaptation factors. Moreover, this approach can be used to explore the adaptation of any virus to a new host species, provided that the virus can be grown in an appropriate model system. The main limitation of this approach is that laboratory results in cell culture or animal model systems may not translate to viral

[483] Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology* 84: 3134-3146

[484] Yang Y *et al* (2015) Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. Ibid. 89: 9119-9123

[485] Pfefferle S *et al* (2009) Distant relatives of severe acute respiratory syndrome coronavirus and close relatives of human coronavirus 229E in bats, Ghana. *Emerging infectious diseases* 15: 1377-1384

[486] Ge XY *et al* (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538

[487] Baric RS *et al* (1999) Persistent infection promotes cross-species transmissibility of mouse hepatitis virus. *Journal of virology* 73: 638-649

[488] Chen W *et al* (2005) SARS-associated coronavirus transmitted from human to pig. *Emerging infectious diseases* 11: 446-448

adaptation to humans in nature. Additionally, results gleaned from the one or two strains under study may not be conserved in other CoV species.

Another GoF method for studying cross-species adaptation involves "Spike swapping" – that is, targeted genetic modification to replace all or part of the CoV Spike protein, a surface protein that mediates virus entry into cells and is a critical determinant of host restriction, with the Spike protein from another CoV species. These experiments are considered Gain of Function because they are expected to alter host tropism in mammalian species. The purpose of these experiments is two-fold. First, testing the ability of chimeric CoVs to infect various types of cells and animals reveals the breadth of host tropism conferred by a given Spike protein, and comparing the sequences of parental and donated Spike proteins with different host tropism can uncover amino acid residues that mediate host restriction. Second, defining the host tropism of animal CoVs and the number of amino acid changes that are needed to confer the ability to infect human cells provides insight into whether the ability to adapt to new species is a conserved feature of CoVs, as well as which animal CoVs are poised to spill over into human populations. Third, because most bat CoVs cannot be cultured in standard cell culture systems, "Spike swapping" enables the chimeric bat-SARS virus to infect and replicate within human cells, thereby enabling further study of the behavior of the bat CoV. The main drawback of this approach is that it is limited to studying the role of the Spike-receptor interaction in host tropism. Another drawback is that chimeric "SARS plus animal CoV Spike" viruses may behave differently from wild type animal CoVs.

A third GoF approach involves serial passaging of bat CoVs in cell culture, which selects for viruses that are better able to bind, infect, and replicate within human cells (i.e., enhanced pathogen production). For those bat CoVs that can infect cells but grow poorly in cell culture, this enables the development of higher-yield viruses that can be used as tools for the study of bat CoV behavior. Understanding the characteristics of bat CoVs relative to human epidemic CoVs may provide insight into the adaptive changes that facilitate efficient infection of humans.

Finally, targeted genetic modification of wild type viruses to introduce mutations that are associated with adaptation to new hosts demonstrates that such markers are *necessary* and *sufficient* to broaden or alter host tropism. This information provides a strong foundation for follow-up studies investigating the mechanistic basis of the adaptation phenotype.

9.3.3.1.2 Scientific Knowledge Benefit 2: Gain Insight into the Mechanisms Underlying the Pathogenicity of CoVs

Why SARS and MERS coronaviruses cause severe respiratory infections while other human coronaviruses cause mild to moderate illness is unknown.[489] Specifically, the viral genetic and phenotypic traits underlying the enhanced pathogenicity of SARS and MERS relative to other human coronaviruses are poorly understood, and only a few viral virulence factors have been identified and characterized (such as the CoV Spike protein, which mediates viral entry into host cells).

Serial passaging of CoVs in cell culture or laboratory animals, which selects for enhanced fitness (*in vitro*) or enhanced virulence (*in vivo*), is a GoF approach that can yield information that addresses this scientific knowledge gap. This approach enables the identification of mutations associated with enhanced fitness/virulence, which can lead to the discovery of new viral virulence factors and provides a foundation for follow-up studies investigating the mechanistic basis of the enhanced fitness/virulence phenotype observed in emergent viruses. A key benefit of this approach is the ability to generate and identify novel mutations and viral proteins that contribute to fitness/virulence, without prior knowledge about viral virulence factors. Moreover, this approach can be performed with any coronavirus that is capable of

[489] (2015i) Interviews with coronavirus researchers

infecting appropriate cell culture or animal model systems. The main drawbacks of serial passaging experiments are that insights may not translate to human infections, and viral factors and phenotypes that contribute to virulence in the CoV strain under study may not generalize to other CoV strains.

A second GoF approach for studying virulence involves targeted genetic modification of wild type viruses to introduce mutations that are associated with enhanced fitness/virulence, which demonstrates that such markers are *necessary* and *sufficient* to enhance fitness/virulence. This information provides a strong foundation for follow-up studies investigating the mechanistic basis of the enhanced virulence phenotype.

9.3.3.1.3 Scientific Knowledge Benefit 3: Gain insight into Disease Pathogenesis, Including Host Factors That Contribute to Disease Pathology

The host factors involved in SARS and MERS pathogenesis are poorly understood. That is, the contribution of host immune responses to the exacerbated pathology observed during infection with SARS-CoV and MERS-CoV relative to the "common cold" human CoVs is unknown.
Animal-adapted viruses, generated through serial passaging of CoVs in mice to enhance their capacity to infect and cause disease in mice (i.e., altered host range and enhanced virulence) are essential tools for the study of CoV pathogenesis. Infection of mice with animal-adapted viruses recapitulates disease pathology observed during human infection, which is critical for studying the mechanisms underlying disease pathology. Many different experimental methods can be used to study disease pathology using mouse models, including characterizing the host immune response to CoV infection, knocking out or depleting specific host immune factors to probe their role in pathogenesis, and analyzing the tissue tropism and dissemination of CoVs over the course of infection. Of note, mouse-adapted viruses are also important for the study of viral genetic and phenotypic traits that contribute to pathogenesis (scientific knowledge gap 2). The main drawback of using mouse-adapted viruses is that adaptive changes may alter the biology of the virus, such that findings are mis-representative of wild type virus behavior.

9.3.3.2 Benefits and Limitations of GoF to Surveillance

Currently, GoF approaches do not have the potential to benefit public health, agricultural animal, or wildlife surveillance. Although CoV researchers stated that they could envision using information about the molecular determinants of human adaptation and virulence to assess the risk posed by animal CoVs circulating in nature, similar to the influenza field, this application is currently unfeasible for two reasons: (1) CoV surveillance networks are extremely limited, with large gaps in coverage in humans and animals, and (2) the state of knowledge about the molecular determinants of human adaptation and virulence is poor.[490]

9.3.3.3 Benefits and Limitations of GoF to Vaccine Development

Currently, there are no FDA-approved vaccines for CoVs, which represents a critical gap in public health preparedness for CoV outbreaks. Several GoF approaches have the potential to benefit the development of new CoV vaccines.

9.3.3.3.1 Vaccine Development Benefit 1: Developing Vaccine Candidates

GoF approaches have the potential to benefit two aspects of the development of live attenuated vaccine (LAV) platforms, which is a type of vaccine that is being actively researched for its potential as a CoV vaccine platform. First, GoF approaches can inform the development of candidate LAV strains, which exhibit attenuated virulence relative to parental strains. Specifically, one strategy for generating LAV

[490] For example, out of more than 1700 bat species, only ten have been surveilled for evidence of CoV infection (and those ten on an ad hoc rather than a systematic basis).

strains is through serial passaging in a non-human host (either an animal or cells derived from an animal), as adapting a virus to a new host typically attenuates the virus in humans (i.e., alters rather than enhances host tropism). Because this approach **alters host tropism**, it is considered to be a GoF approach under the NSABB Framework. Although serial passaging has been used historically for developing polio, smallpox and other viral vaccines, the approach has not been utilized for the purpose of developing CoV vaccine strains.[491] Alternatively, live attenuated vaccines can be generated through targeted mutagenesis to attenuate or knock out the function of known virulence factors. As described above (Section 9.3.3.1.2), GoF studies that **enhance virulence** represent the most efficient and effective strategy for identifying novel CoV virulence factors, which may be good targets for attenuation to develop an LAV. However, follow-up studies are needed to determine how to attenuate that factor or to render it non-functional.

LAVs are an appealing type of vaccines for CoVs for several reasons, and multiple LAV candidates for SARS have been shown to protect against lethal virus challenge in mice, demonstrating the promise of this type of vaccine for CoVs.[492,493] However, a major concern is their potential to regain virulence in people, which necessitates stringent safety testing of all LAV candidates.

9.3.3.3.2 Vaccine Development Benefit 2: Determining the Potential for LAVs to Recover Virulence

Once a candidate LAV strain has been generated, the strain is typically serially passaged *in vitro* or *in vivo* to determine whether the virus recovers fitness/virulence (i.e., **enhanced fitness/virulence**). Because a tendency to revert or acquire compensatory mutations that enhance fitness/virulence could seriously compromise the safety of a live attenuated vaccine, demonstrating the genetic stability of a candidate LAV is a critical aspect of its development.

9.3.3.4 Benefits and Limitations of GoF to Therapeutic Development

Currently, there are no FDA-approved therapeutics for CoVs, which represents a critical gap in public health preparedness for CoV outbreaks. Several GoF approaches have the potential to benefit the development of new CoV therapeutics.

9.3.3.4.1 Therapeutic Development Benefit 1: Developing Candidate Therapeutics

CoV researchers cited the lack of knowledge of good viral targets for therapeutics as a critical limitation for the development of CoV therapeutics.[494] GoF approaches currently represent the most efficient and effective way to identify novel virulence factors and gain insight into their mechanism of activity, a foundation for the development of antivirals (see Section 9.3.3.1.2). However, whether inhibiting or attenuating the virulence factor is sufficient to reduce viral replication and infection-associated pathology must be determined through alternative approaches.

9.3.3.4.2 Therapeutic Development Benefit 2: Generating Nonclinical Data to Support an Investigational New Drug Application to the FDA

The first step in the licensure process for new drugs involves submission of an Investigational New Drug (IND) application to the FDA's Center for Drug Evaluation and Research (CDER). CDER recommends that several types of nonclinical studies are conducted before starting Phase I clinical studies, including

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491 Ulmer JB *et al* (2006) Vaccine manufacturing: challenges and solutions. *Nature biotechnology* 24: 1377-1383

492 Graham RL *et al* (2012) A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. *Nature medicine* 18: 1820-1826

493 Fett C *et al* (2013) Complete protection against severe acute respiratory syndrome coronavirus-mediated lethal respiratory disease in aged mice by immunization with a mouse-adapted virus lacking E protein. *Journal of virology* 87: 6551-6559

494 (2015i) Interviews with coronavirus researchers.

determination of the drug's mechanism of action, *in vitro* selection of resistant viruses to the investigational product, and the genotypic and phenotypic characterization of resistant viruses.[495] GoF approaches that lead to evasion of therapeutics generate information that fulfills both of those recommendations, thereby supporting the licensure of new therapeutics.

First, serial passaging of viruses in the presence of a therapeutic to select for resistant viruses, followed by sequencing of the emergent resistant strains to identify genetic changes that arose, can provide insight into the mechanism of action of the therapeutic. Understanding which viral protein or proteins mutate in order for the virus to escape inhibition suggests those proteins are targeted by the therapeutic, and the site and phenotypic consequences of the mutations may provide insight into the mechanism of antiviral activity. Together, this information provides a foundation for follow-up structural, biochemical, and cell biological assays investigating the mechanism of antiviral activity. Second, this approach directly fulfills FDA's recommendation for *in vitro* selection of resistant viruses, which is performed to determine the genetic threshold for the development of resistance (i.e., the number of mutations that are needed for a virus to acquire resistance).

9.3.3.4.3 Therapeutic Development Benefit 3: Determining the Therapeutic Dosage and/or Combination Therapies That are Least Likely to Lead to the Emergence of Resistance

GoF studies that lead to evasion of therapeutics can also inform the therapeutic dosage and the use of combination therapies, both of which influence whether and how readily antiviral resistance arises. Specifically, serial passaging of virus in animals dosed with varying amounts of the therapeutic provides insight into the dosage that is least likely to lead to the emergence of resistant viruses, and serial passaging of virus in cells or in animals in the presence of multiple mAbs (or other types of therapeutics) can be used to determine how readily resistance arises in response to combination versus single therapies. This information may lead to the development of therapeutic strategies that will be effective for a longer period of time in the field.

9.3.3.5 Benefits and Limitations of GoF to Both Vaccine and Therapeutic Development

9.3.3.5.1 Vaccine/Therapeutic Development Benefit 1: Testing the Safety and Efficacy of MCM Candidates

The use of animal-adapted viruses, generated using GoF approaches that **alter host range** and **enhance virulence**, facilitate MCM development by enabling the testing of MCM candidates in an animal model that mimics the pathology of human disease. Animal-adapted strains represent a robust system for demonstrating that a candidate MCM is capable of preventing or reducing disease-associated pathology. In addition, the use of models that share features of human disease can reveal adverse side effects of the vaccine and thus is an important aspect of safety testing prior to the initiation of human clinical trials.

9.3.3.5.2 Vaccine/Therapeutic Development Benefit 2: Developing Broad-Spectrum Vaccines

Finally, GoF approaches that **alter host range** inform the development of broad-spectrum vaccines that may be capable of protecting against the next emerging CoV. Specifically, chimeric bat-SARS viruses can be used as challenge viruses to explore the broad-spectrum potential of candidate MCMs, in order to test whether MCMs designed to target SARS/MERS proteins are also capable of targeting cognate proteins in bat CoVs as well as whether vaccines can target SARS/MERS proteins in a different virus

[495] Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

context (representative of the next emerging CoV capable of infecting humans). These experiments can provide insight into whether MCMs targeting any CoV protein or process are capable of conferring broad-spectrum protection against bat CoVs with zoonotic potential, in addition to SARS and MERS.

9.3.3.6 Benefits and Limitations of GoF to Diagnostic Development

As diagnostic targets for CoVs are well-established, potential benefits of GoF approaches to the development of diagnostics were not identified.[496, 497,498, 499]

9.3.3.7 Benefits and Limitations of GoF to Decision-Making in Public Health Policy

Because the US government is not actively engaged in public health preparedness activities for CoV outbreaks and because there are no FDA-approved vaccines or therapeutics for CoVs, GoF approaches do not have the potential to benefit decision-making in public health policy (e.g., informing countermeasure stockpiling decisions, guiding decisions about strain selection for vaccine development, etc.)

9.3.3.8 Economic Benefits

GoF benefits to the development of vaccines and therapeutics could have downstream economic benefits. Economic benefits were not explicitly evaluated in this report.

9.3.4 Identification of Alt-GoF That Provide Similar Potential Benefits to the GoF Being Examined

In this section, an overview of alternative (alt-GoF) approaches that yield the same or similar benefits as the GoF approaches described above is provided. Two types of alt-GoF approaches are reviewed: (1) alternative experimental approaches that can provide the same or similar scientific information as GoF experimental approaches, and (2) alternative scientific and technical innovations that can yield the same public health benefits as GoF approaches but through different mechanisms, including the use of alternative model systems that do not rely on GoF approaches. For each approach, the scientific outcomes or products of the approach are first described, then how that information or products leads to similar benefits as GoF approaches.

9.3.4.1 Benefits and Limitations of Alt-GoF Approaches to Scientific Knowledge

9.3.4.1.1 Scientific Knowledge Benefit 1: Gain Insight into the Mechanisms Underlying Adaptation of Animal CoVs to Humans

Several alternative experimental approaches can be used to discover genetic traits associated with cross-species adaptation of CoVs.

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[496] The FDA-approved diagnostic test for MERS-CoV targets two regions in the CoV genome: a region upstream of the E gene (*upE*) and the reading frame 1a (*orf1a*). SARS can be detected through RT-PCR with sequences in the polymerase 1 B region (*pol* 1B) and an adjacent downstream region of the genome as the targets. Other diagnostic tests target sequences in the nucleocapsid (N) gene.

[497] Stephen M. Ostroff Acting Commissioner of Food and Drugs. Letter of Authorization RealStar® MERS-CoV RT-PCR Kit U.S. . http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM455348.pdf. Last Update July 17, 2015. Accessed December 2015.

[498] Richardson SE *et al* (2004) The laboratory diagnosis of severe acute respiratory syndrome: emerging laboratory tests for an emerging pathogen. *The Clinical biochemist Reviews / Australian Association of Clinical Biochemists* 25: 133-141

[499] Mahony JB *et al* (2004) Performance and Cost evaluation of one commercial and six in-house conventional and real-time reverse transcription-pcr assays for detection of severe acute respiratory syndrome coronavirus. *J Clin Microbiol* 42: 1471-1476

Comparing the sequences of CoVs with different species tropism, including comparison of animal CoVs versus SARS/MERS and comparison of animal strains from different geographic regions where spillover into human populations has and has not occurred (or has occurred with different frequencies), can elucidate genetic traits that are associated with adaptation to different hosts. Second, comparative sequence analysis of human CoVs from different time points during an outbreak reveals how zoonotic CoVs adapt to humans following an initial spillover event. Relative to the laboratory methods described above, this approach has potential to identify traits that are relevant for adaptation to humans under natural selective pressures. Importantly, follow-up studies are needed to confirm that the identified genetic traits are responsible for altered host tropism.

Both types of comparative sequence approaches suffer from several significant limitations. First, the success of comparative sequence analysis is significantly constrained by the quality and availability of existing genetic surveillance data. A second limitation is that, due to the large size of the CoV genome (27-32 kb) and the genetic diversity of coronaviruses in nature, there are a very large number of genetic differences between any two CoV strains, only a subset of which are likely to be important for cross-species adaptation.[500] Because of that "noise," sequence comparisons are realistically limited to known regions of interest, precluding discovery of novel factors that are involved in host adaptation. Due to the fact that only a few proteins have been shown to be involved in cross-species adaptation and the function of most CoV proteins is unknown, this limited focus represents a critical shortcoming of the comparative sequence analysis approach. Although this limitation could be partially addressed by comparing sequences of paired animal and human isolates, few such paired sequences are available. Third, this approach is reactive, limited to the study of mechanisms underlying adaptation of CoVs that have already evolved to broaden or alter their host tropism (e.g., SARS and MERS). The mechanisms driving adaptation of other CoVs to new hosts may be different.

Several alternative approaches seek to define the breadth of host tropism conferred by a given Spike protein. The first approach involves testing whether MERS- or SARS-CoVs can infect cells derived from various non-human host species such as bats or cells that do not naturally express CoV receptor proteins but have been engineered to ectopically express receptor proteins from various species. This approach cannot be used for most animal CoVs, which cannot be grown efficiently in cell culture to produce infectious material for laboratory assays. Alternatively, two virus-free approaches can provide information about compatible Spike-host interactions: (1) *in vitro* binding assays using recombinant Spike proteins and host receptor proteins from different species, and (2) cell culture-based binding and virus entry assays using non-CoVs (e.g., murine leukemia virus) that are pseudotyped with CoV Spike proteins. (Pseudotyping is the process of expressing the envelope protein or surface glycoprotein from one virus on the surface of a different virus, e.g., replacement of the vesicular stomatitis virus glycoprotein (VSV G) with the CoV Spike, enabling expression of the CoV Spike on the surface of VSV.) These *in vitro* systems can also be used to confirm that amino acid substitutions in the Spike protein are necessary and sufficient to alter host receptor binding and cell entry capabilities. The major limitation associated with these virus-free approaches is that results may not be recapitulated in the context of the wild type virus, as the virus context influences presentation of surface epitopes. Additionally, results from either virus-free approach may not be conserved in a different strain context, and traits that promote binding of pseudotyped viruses to a particular cell type may not be critical for adaptation to human hosts. Finally, these approaches are currently used to investigate the role of the Spike-receptor interaction in host restriction only.

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[500] Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology* 84: 3134-3146

Finally, structural modeling of Spike-receptor interactions, based on crystal structures of Spike-receptor complexes, can also be used to identify amino acid residues in the Spike protein that may be important determinants of host restriction. Though useful for generating hypotheses about mutations that may alter host tropism, all predictions must be experimentally confirmed.

In addition to the use of serially-passaged bat CoVs or chimeric CoVs, several alternative model systems can be used to study the biology of bat CoVs, which may provide insight into the adaptive changes that are needed for CoVs to efficiently infect humans. First, some bat CoVs are naturally capable of replicating within bat cell lines or other standard cell culture systems. However, bat cell lines are much less experimentally tractable than human cell lines, as fewer reagents are available and the cells are more difficult to transfect than human cells, further lessening the utility of this approach.[501,502] Second, host cells that are not naturally permissive to infection with animal CoVs can be sensitized to infection through ectopic expression of the receptor protein from the natural host species (or another permissive host species). This approach has been utilized for a limited number of CoVs, and whether it will permit replication of a broad range of emerging CoVs is unknown. Furthermore, this strategy cannot be used for primary cell lines, which are not readily transfectable, and overexpression of the receptor may alter the process of infection, leading to artefactual results.

9.3.4.1.2 Scientific Knowledge Benefit 2: Gain Insight into the Mechanisms Underlying the Pathogenicity of CoVs

Several alternative approaches can also be used to study pathogenicity. Comparative sequencing of SARS-CoV and MERS-CoV epidemic strains with varying levels of virulence can lead to the identification of mutations associated with enhanced virulence. A strength of this approach relative to serial passaging is that comparative sequence analysis uncovers genetic variation that is specially associated with enhanced virulence in humans.[503,504] However, this approach is limited to CoVs that have already produced epidemics in humans, i.e., SARS-CoV and MERS-CoV. The success of this approach is constrained by the quality and availability of surveillance data, in particular the quality of "metadata" about clinical severity that is needed to "bin" sequences into low- and high-virulence categories for comparison. While SARS-CoV strains from the early, middle, and late phases of the 2002 – 2003 epidemic have been found to exhibit varying levels of virulence (and have been used for comparative sequence analysis studies), genetic surveillance data for MERS are limited. Finally, given the large size of the CoV genome and genetic diversity among wild type CoV sequences, sequence comparisons are practically limited to pre-determined regions of interest, which precludes identification of novel virulence factors.

A second sequence-based approach involves analyzing the evolution of CoVs over time. Understanding which regions of the genome mutate and which do not can provide insight into which regions are likely to be critical for the virus life cycle, which may or may not contribute to pathogenicity. However, the utility of this approach is also limited by the number of available CoV sequences.

Loss of Function (LoF) studies, which involve knocking out or otherwise hampering the function of a gene of interest (or its product) and screening for attenuated fitness (*in vitro*) or virulence (*in vivo*), represent another alternative approach for the discovery of viral virulence factors and genetic traits

501 Yang Y *et al* (2015) Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. Ibid. 89: 9119-9123

502 Huynh J *et al* (2012) Evidence supporting a zoonotic origin of human coronavirus strain NL63. Ibid. 86: 12816-12825

503 de Jong MD *et al* (2005) Oseltamivir Resistance during Treatment of Influenza A (H5N1) Infection. *New England Journal of Medicine* 353: 2667-2672

504 Chinese SMEC (2004) Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science* 303: 1666-1669

associated with virulence. Given the large size of the CoV genome, a random mutagenesis approach is practically limited to the investigation of known virulence factors. A targeted gene knockout strategy can be used to identify new viral genes that contribute to virulence, but a limited number of mutants can be screened for attenuated virulence *in vivo*, due to the labor, expense, and ethical considerations associated with the conduct of animal experiments. Thus, high-throughput screening of gene knockout viruses is limited to screening for attenuated fitness in cell culture systems, which is only one aspect of virulence. The major drawback of LoF screens is that losing the functionality of a virus protein, either through gene knockout of mutagenesis, may indirectly attenuate virulence, so that gaining meaningful information about virulence mechanisms may be difficult using this approach. Finally, it is noted that knocking out the function of an unknown viral protein can lead to a loss or gain of virulence, depending on the function of the protein.

LoF approaches can also be used to confirm that a particular trait is *necessary* for enhanced virulence. However, because virulence is a complex, multi-genic trait, knocking out the function of one gene or introducing a mutation into one gene may be sufficient to attenuate virulence but provides an incomplete picture of the role of that particular protein. Additionally, mutations that are found to enhance virulence in model systems may not translate to increased virulence during human infections.

9.3.4.1.3 Scientific Knowledge Benefit 3: Gain Insight into Disease Pathogenesis, Including Host Factors That Contribute to Disease Pathology

In addition to using animal-adapted viruses generated through GoF approaches, several alternative model systems can be used to study disease pathogenesis.

Naturally susceptible laboratory animals represent one alternative model system for studying disease pathogenesis. However, laboratory animals that are naturally susceptible to infection with SARS-CoV and MERS-CoV have been found to support viral replication but remain asymptomatic or develop symptoms dissimilar to those in humans. Thus, these animal models are not suitable for pathogenesis studies.

Use of transgenic animals expressing the human virus receptor is another alternative to the use of adapted viruses for hosts that are not permissive to infection or do not recapitulate human disease pathology. A variety of approaches have been used to create transgenic mouse models for SARS-CoV and MERS-CoV infection, and each technique results in a slightly different gene expression pattern and reproduces human disease symptoms to a different degree. The ability to infect transgenic mice with wild type SARS-CoV and MERS-CoV is a strength of this model system. However, given differences in pathogenesis, results may not translate to human disease.

Finally, human autopsy data can be an alternative source of pathogenesis information. However, the availability of these data are limited – autopsies are not often performed in Middle Eastern cultures, and data has not yet been shared from the most recent outbreak in the Republic of Korea.[505] Furthermore, analysis of human autopsy data provides limited mechanistic insight because it is inherently correlative and is devoid of time series information, obscuring the order in which pathogenic effects occurred. Additionally, insights gleaned from the study of severe, end-stage disease may not be representative. The fact that many SARS-CoV and MERS-CoV deaths occurred in patients with pre-existing conditions further complicates the identification of pathology caused by viral infection versus comorbidities.

[505] (2015i) Interviews with coronavirus researchers.

9.3.4.2 Benefits and Limitations of Alt-GoF Approaches to Vaccine Development

9.3.4.2.1 Vaccine Development Benefit 1: Developing Vaccine Candidates

Live attenuated vaccines (LAVs) may be generated through targeted mutagenesis to knock out or attenuate the function of known virulence factors. LoF approaches, namely screening of gene knockout viruses or randomly mutagenized viruses for attenuated virulence, are relatively inefficient for the discovery of novel virulence factors but can be used to confirm that inhibiting or attenuating the function of a virulence factor is sufficient to attenuate virus replication.

In addition to LAVs, several other types of CoV vaccines are in development, which do not rely on GoF approaches for their initial development. Alternative vaccine platforms of interest include inactivated whole virus vaccines, recombinant vaccines, DNA vaccines, viral vector-based vaccines, and virus-like particles (VLPs).[506] Many of these vaccine types have shown promise, and each has strengths and limitations relative to the use of live attenuated vaccines.

9.3.4.2.2 Vaccine Development Benefit 2: Determining the Potential for LAVs to Recover Virulence.

There are no alternative approaches for determining the potential for LAVs to recover virulence upon growth in cells or animals prior to the clinical testing of vaccine candidates in people.

9.3.4.3 Benefits and Limitations of Alt-GoF Approaches to Therapeutic Development

9.3.4.3.1 Therapeutic Development Benefit 1: Developing Candidate Therapeutics

Several alternative approaches can inform the development of candidate therapeutics against CoVs. First, LoF approaches can lead to the identification of novel virulence factors, which may be good targets for new therapeutics. LoF approaches are relatively inefficient for the discovery of novel virulence factors but are critical for demonstrating that inhibition or attenuation of a virulence factor is sufficient to reduce viral replication or infection-associated pathology.

An alternative approach to the targeted development of therapeutics involves high-throughput screening of compounds for their ability to reduce viral replication *in vitro*.[507,508,509,510,511] This is also an active area of therapeutic research in the CoV field and has generated several promising candidates. One drawback of this approach is that it is limited to the identification of compounds that reduce viral replication, which is only one aspect of virulence. Targeting other aspects of virulence, such as viral interactions with the host immune system, may prove to be a more effective therapeutic strategy.

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506 Zhang N *et al* (2014) Current advancements and potential strategies in the development of MERS-CoV vaccines. *Expert Rev Vaccines* 13: 761-774

507 de Wilde AH *et al* (2014) Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrobial agents and chemotherapy* 58: 4875-4884

508 Dyall J *et al* ibid.Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. 4885-4893

509 Ratia K *et al* (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences of the United States of America* 105: 16119-16124

510 Wu CY *et al* (2004) Small molecules targeting severe acute respiratory syndrome human coronavirus. Ibid. 101: 10012-10017

511 Severson WE *et al* (2007) Development and validation of a high-throughput screen for inhibitors of SARS CoV and its application in screening of a 100,000-compound library. *Journal of biomolecular screening* 12: 33-40

A related alternative approach involves high-throughput screening of panels of monoclonal antibodies (mAbs) to identify mAbs that bind to CoV Spike proteins, as mAbs targeting the Spike protein have been shown to effectively prevent viruses from infecting cells and could prime the immune system to clear the infection.[512] One potential drawback of this therapeutic strategy is that CoVs can readily acquire mutations in their Spike protein that enable escape from mAb neutralization; however, researchers are actively pursuing the development of "cocktails" of mAbs that are more robust to the generation of escape mutants.[513,514] Additional drawbacks are that antibody-based therapeutics, which are uncommon for infectious diseases, may only slow infections and must be injected because antibodies are not small molecules.

9.3.4.3.2 Therapeutic Development Benefit 2: Generating Nonclinical Data to Support an Investigational New Drug Application to the FDA

Several alternative approaches can be used to investigate the mechanism of activity of a new therapeutic candidate. First, high-throughput RNAi screens targeting host proteins can identify host proteins that are required for the drug's mechanism of action, by demonstrating that knockdown of a particular host protein impedes the drug's ability to inhibit viral replication. Though an informative strategy for the study of therapeutics targeting host proteins, high-throughput RNAi screens provide minimal information about potential viral targets of therapeutics. (It should be noted that this approach is typically performed to identify the potential targets of drugs identified through high-throughput screens, as the candidate drugs may attenuate viral replication by directly targeting viral proteins or by indirectly targeting host proteins.)

If the therapeutic target of a drug is known, analyzing the crystal structure of the viral target in complex with the antiviral compound (or mAb) can provide insight into the compound's mechanism of activity.[515,516] This approach is particularly useful for therapeutics that directly bind to and inhibit the activity of a viral protein. Though X-ray crystallography is appealing for its potential to provide direct information about the interaction between an antiviral and its target, inferring how that interaction affects a process in the viral life cycle may be difficult from such a static snapshot. Critically, because of the high level of effort required for X-ray crystallography, it is not a feasible approach for simply screening the potential viral targets of an unknown antiviral.

Photoaffinity cross-linking represents an alternative approach for identifying the binding site of a drug with a known target. This technique shares strengths and weaknesses with X-ray crystallography. Namely, photoaffinity cross-linking is useful for small molecule drugs that directly bind to and inhibit the activity of a viral protein and does not require prior knowledge of the location of the drug binding site.[517] However, inferring the mechanism of antiviral activity based on knowledge about the drug-virus protein interaction may be difficult.

There are no alternative approaches that can determine the genetic threshold for resistance to a new therapeutic, which is a recommended piece of data to support an Investigational New Drug (IND)

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512 Sui J *et al* (2008) Broadening of neutralization activity to directly block a dominant antibody-driven SARS-coronavirus evolution pathway. *PLoS pathogens* 4: e1000197

513 Rockx B *et al* (2010) Escape from human monoclonal antibody neutralization affects in vitro and in vivo fitness of severe acute respiratory syndrome coronavirus. *The Journal of infectious diseases* 201: 946-955

514 Sui J *et al* (2014) Effects of human anti-spike protein receptor binding domain antibodies on severe acute respiratory syndrome coronavirus neutralization escape and fitness. *Journal of virology* 88: 13769-13780

515 Prabakaran P *et al* (2006) Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. *The Journal of biological chemistry* 281: 15829-15836

516 Ratia K *et al* (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences of the United States of America* 105: 16119-16124

517 Hamouda AK *et al* (2014) Photoaffinity labeling of nicotinic receptors: diversity of drug binding sites! *Journal of molecular neuroscience : MN* 53: 480-486

application to the FDA, prior to deployment of the therapeutic and the emergence of resistant viruses in nature.

9.3.4.3.3 Therapeutic development benefit 3: Determining the therapeutic dosage and/or combination therapies that are least likely to lead to the emergence of resistance

No alternative approaches are capable of providing information about the dose-dependence of resistance or whether combination therapies lead to resistance less readily than individual therapies, prior to clinical testing or post-marketing studies.

9.3.4.4 Benefits and Limitations of Alt-GoF Approaches to Both Vaccine and Therapeutic Development

9.3.4.4.1 Vaccine/Therapeutic Development Benefit 1: Testing the Safety and Efficacy of MCM Candidates

In addition to animal-adapted viruses, several alternative model systems could be used to test the safety and efficacy of vaccine candidates, namely naturally susceptible hosts and transgenic animals (see Section 9.3.4.1.3). Transgenic mice are important in countermeasure development because they can be used to establish that a therapy knocks down virus titers in a system with human receptors.[518] However, the predictive value of safety and efficacy data gleaned from experiments using transgenic animals is constrained by the fact that transgenic animals do not fully recapitulate human disease pathogenesis. Naturally susceptible hosts of SARS or MERS are either asymptomatic or develop symptoms dissimilar to those in humans. As a result, these "replication" models have limited utility for advanced vaccine development. Replication models may provide easy metrics to demonstrate vaccine or drug efficacy (i.e., reduction in viral replication), but their lack of relevant symptomology could lead to the development and release of subpar or dangerous countermeasures. Specifically, therapeutics may cause unintended side effects or deleterious interactions with the host immune system, which are unpredictable and may not be observed in asymptomatic animal models.

9.3.4.4.2 Vaccine Development Benefit 4: Developing Broad-Spectrum Vaccines

In addition to chimeric bat-SARS viruses generated through GoF approaches, several alternative model systems can be used to evaluate the broad-spectrum potential of candidate MCMs. One approach involves the use of wild type bat CoVs as challenge viruses, in lieu of chimeric bat-SARS viruses. However, the fact that few bat CoVs can be grown in culture or in animals without the use of GoF approaches (serial passaging or the generation of chimeric viruses) diminishes the utility of this approach.
For evaluating MCMs that target the Spike protein, the use of pseudotyped viruses represents another alternative approach. Because Spike proteins are presented differently in the context of pseudotyped viruses versus CoVs, results using pseudotyped viruses may not be recapitulated in the context of the wild type virus.[519] Thus, all results using pseudotyping systems must be confirmed using wild type viruses (or chimeric CoVs, which better mimic wild type bat CoVs than pseudotyped viruses). Finally, chimeric viruses that have been engineered to express "internal" (i.e., non-Spike) CoV proteins have been used for testing the efficacy of therapeutics targeting non-Spike proteins.[520] As with pseudotyped viruses, due to significant differences in the course of infection between chimeric virus systems and wild type viruses, such chimeric virus systems can be used to screen therapeutic candidates but do not replace the need to test MCMs against the wild type virus.

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518 (2015i) Interviews with coronavirus researchers.
519 Ibid.
520 Deng X *et al* (2014) A chimeric virus-mouse model system for evaluating the function and inhibition of papain-like proteases of emerging coronaviruses. *Journal of virology* 88: 11825-11833

9.3.5 Comparison and Analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

9.3.5.1 Benefits to Scientific Knowledge

9.3.5.1.1 Scientific Knowledge Benefit 1: Gain Insight into the Mechanisms Underlying Adaptation of Animal CoVs to Humans

Serial passaging, a GoF approach that alters host range, is **uniquely capable** of identifying *novel* viral genetic traits and factors that contribute to cross-species adaptation. Moreover, to elucidate the molecular mechanisms underlying the role of the Spike-receptor interaction in host adaptation, testing the phenotypic consequences of mutations in animal CoV Spike proteins in the context of a chimeric virus generated through GoF approaches provides a higher level of certainty in the validity of the results than similar confirmatory experiments using recombinant proteins or pseudotyped viruses. However, sequence comparisons, an alt-GoF approach, are uniquely capable of identifying genetic traits that are associated with mammalian adaptation across a variety of strains as well as discovering genetic markers that are definitively associated with human adaptation. However, the causality of markers identified through sequence analysis must be confirmed with a GoF experiment, and the utility of the comparative sequence approach is severely compromised by the poor state of genetic surveillance for CoVs in human and animal populations and the fact that it is limited to analysis of strains that have caused human infections.

In addition to the approaches described above, characterizing SARS/MERS-like animal CoVs, thought to be precursors for SARS/MERS or to have similar potential to spill over into human populations, also provides insight into how SARS and MERS emerged from their animal reservoirs to infect humans. However, most animal CoVs grow poorly, if at all, in standard cell culture systems. GoF approaches have **unique potential** to enable the development of *in vitro* model systems for the study of any animal CoV in a variety of cell types, including immortalized cell lines and relevant primary cell lines such as human epithelial airway cells. Alternatives to GoF have significant shortcomings. Only a subset of animal CoVs identified to date can be cultured in bat, human, or other standard cell lines, limiting the utility of using naturally permissive cell lines for *in vitro* studies. While ectopic expression of permissive receptor proteins in a common cell line has been shown to permit replication of several CoVs, this strategy is limited to cell lines that can be readily transfected (i.e., not primary cell lines) and overexpression of the host receptor may alter the biology of infection, limiting the relevance of results from this system.

9.3.5.1.2 Scientific Knowledge Benefit 2: Gain Insight into the Mechanisms Underlying the Pathogenicity of CoVs

Serial passaging for the selection of CoV strains with enhanced pathogenicity in animals or fitness in cell culture, a GoF approach, is the most efficient and effective method for identifying novel genetic traits and/or viral factors that contribute to virulence in any coronavirus strain. The alternate approaches have several drawbacks. While screening gene knockout viruses *in vitro* represents a viable approach for the discovery of novel virulence factors, this LoF approach is limited to the identification of proteins that influence replicative fitness, only one component of virulence, and may uncover factors that attenuate virulence for trivial reasons. The main drawback of both the GoF and LoF approaches is that insights gleaned from model systems may not translate to human infection. To that end, comparatively analyzing the sequences of SARS/MERS strains with varied levels of virulence can provide direct insight into genetic traits that are associated with pathogenicity in humans. However, this approach is limited to the study of SARS and MERS and is significantly constrained by shortcomings in the quality and availability of existing genetic surveillance data. In addition, any hypothesis generated through comparative sequence analysis must be experimentally confirmed. The phenotypic consequences of mutations that are associated with enhanced virulence can be validated using GoF approaches, which are uniquely capable

of demonstrating that mutations are necessary and sufficient to enhance virulence, or LoF approaches, which can demonstrate that mutations are necessary for enhanced virulence only. Complex, multi-genic traits such as virulence are difficult to tease apart using solely LoF approaches because LoF provides limited information about how proteins cooperate to enhance virulence. However, because the value of the information gleaned from both LoF and GoF approaches depends on the relevance of artificially manipulated viruses to nature, using both approaches to confirm the role of a particular mutation or phenotype strengthens any conclusion.

9.3.5.1.3 Scientific Knowledge Benefit 3: Gain Insight into Disease Pathogenesis, Including Host Factors That Contribute to Disease Pathology

Mouse-adapted strains of SARS, which exhibit altered host range and enhanced virulence in mice relative to the wild type SARS virus, represent the only model system that recapitulates disease pathogenesis observed during human infections of SARS-CoV. As existing animal models for MERS-CoV do not replicate human disease pathology, mouse-adapted strains of MERS-CoV are expected to serve as the sole pathogenesis model for the study of MERS-CoV infection as well. As such, animal-adapted strains can be used to study many facets of disease pathogenesis, including the course of disease, the role of viral and host immune factors in disease pathology, and the role of tissue tropism in disease pathology. Alternative model systems have critical drawbacks for the study of disease pathogenesis. Because transgenic animals do not recapitulate the features of human disease, lessons learned about pathogenesis may not translate to humans. Most naturally susceptible hosts are asymptomatic or display dissimilar symptoms to humans and thus cannot be used to study disease pathogenesis. While human autopsy data are uniquely capable of providing insight into human disease pathology, limited autopsy data are available, and the static nature of the data and the presence of co-morbidities in many SARS/MERS patients complicate interpretation of that data.

9.3.5.2 Benefits to the Development of Vaccines

9.3.5.2.1 Vaccine Development Benefit 1: Developing Vaccine Candidates

Live attenuated vaccines (LAVs) are being actively researched for their potential as CoV vaccine platforms. GoF approaches that enhance virulence represent the most efficient and effective strategy for identifying CoV virulence factors, which may be good targets for attenuation. LoF approaches are relatively inefficient for the discovery of novel virulence factors but are critical for demonstrating that mutagenesis or knockout of a particular virulence factor is sufficient to attenuate viral replication, the goal of generating an LAV candidate.

LAVs are an appealing type of vaccine for CoVs for several reasons, and multiple LAV candidates for SARS have been shown to completely protect against lethal virus challenge in mice, demonstrating the promise of this type of vaccine for CoVs.[521,522] However, one significant concern associated with LAVs is their potential to regain virulence in people. Several other types of CoV vaccines are in development, which do not rely on GoF approaches, and many have shown promise in animal models. Each alternative vaccine type has strengths and weaknesses relative to LAVs, and the type or types of vaccines that will ultimately prove to be most effective for SARS, MERS, and SARS/MERS-like coronaviruses are not yet clear based on vaccinology research conducted to date.[523] Given the need for CoV vaccines, pursuing all

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[521] Graham RL *et al* (2012) A live, unpaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. *Nature medicine* 18: 1820-1826

[522] Fett C *et al* (2013) Complete protection against severe acute respiratory syndrome coronavirus-mediated lethal respiratory disease in aged mice by immunization with a mouse-adapted virus lacking E protein. *Journal of virology* 87: 6551-6559

[523] Zhang N *et al* (2014) Current advancements and potential strategies in the development of MERS-CoV vaccines. *Expert Rev Vaccines* 13: 761-774

promising strategies for vaccine development in tandem, including LAVs, will ensure that an effective vaccine is achieved in the shortest possible period of time.

9.3.5.2.2 Vaccine Development Benefit 2: Determining the Potential for LAVs to Recover Virulence.

GoF approaches, namely serial passaging of LAVs in cells or animals, **are uniquely capable** of determining whether a candidate LAV will recover fitness/virulence upon growth in cells or animals. Because a tendency to revert or acquire compensatory mutations that enhance fitness/virulence could seriously compromise the safety of a live attenuated vaccine, demonstrating the genetic stability of a candidate LAV is a critical aspect of its development.

9.3.5.3 Benefits to the Development of Therapeutics

9.3.5.3.1 Therapeutic Development Benefit 1: Developing Candidate Therapeutics

As described above (Section 9.3.5.1.1), GoF approaches represent the most efficient and effective strategy for identifying novel CoV virulence factors, which may serve as good therapeutic targets. However, follow-up studies are needed to develop therapeutics that inhibit the function of that virulence factor and to determine whether blocking its function is sufficient to reduce disease-associated pathology and/or viral shedding. High-throughput screening of small molecule compounds for their ability to block viral replication *in vitro* has generated several promising therapeutic candidates but is limited to the discovery of therapeutics that inhibit viral replication, which is only one aspect of virulence. Several research groups are pursuing the development of monoclonal antibodies targeting the CoV Spike protein, as mAb binding has been shown to inhibit the ability of the virus to bind and infect cells, but mAb-based therapeutics suffer several drawbacks relative to small molecule drugs and other types of therapeutics. As for CoV vaccines, the type or types of therapeutics that will ultimately prove to be effective against CoVs is not yet clear based on current research. Given the need for CoV therapeutics, pursuing all promising strategies for therapeutic development in tandem will ensure that an effective therapeutic is achieved in the shortest possible period of time.

9.3.5.3.2 Therapeutic Development Benefit 2: Generating Nonclinical Data to Support an Investigational New Drug Application to the FDA

Serial passaging of a virus in the presence of therapeutic to discover mutations that confer resistance, a GoF approach, is **uniquely capable** of identifying the viral target of a novel therapeutic with an unknown mechanism of action. For therapeutics with known viral targets, this information about resistance mutations can provide foundational information to guide follow-up structural, cell biological, and biochemical studies investigating the mechanism of action of the therapeutic. Although crystallography and photoaffinity cross-linking can also provide insight into the antiviral mechanisms of therapeutics that directly bind to and inhibit virus proteins, inferring mechanistic information based on static information about the virus-antiviral complex may be difficult. Finally, the identification of host factors that are required for antiviral activity is a critical aspect of examining therapeutics with unknown targets but provides limited mechanistic information about therapeutics that target virus proteins.

Additionally, serial passaging of viruses in the presence of therapeutic is uniquely capable of determining the genetic threshold for resistance development prior to deployment of the therapeutic and the emergence of resistant strains in nature.

As both mechanism of action and selection for resistance studies to determine the genetic threshold for resistance development are recommended components of an Investigational New Drug application to the

FDA, GoF approaches that lead to the generation of viruses that are resistant to therapeutics in development are **essential** for the licensing of new therapeutics.

9.3.5.3.3 Therapeutic Development Benefit 3: Determining the Therapeutic Dosage and/or Combination Therapies that are Least Likely to Lead to the Reemergence of Resistance

GoF approaches that lead to the generation of viruses that are resistant to therapeutics in development are **uniquely capable** of determining the therapeutic dose that is least likely to lead to the acquisition of antiviral resistance as well as determining whether combination therapies better prevent the emergence of resistant viruses than individual therapies. Both types of information benefit the development of therapeutic strategies that will be effective for a longer period of time in the field.

9.3.5.4 Benefits to the Development of Vaccines and Therapeutics

9.3.5.4.1 Vaccine/Therapeutic Development Benefit 1: Testing the Safety and Efficacy of MCM Candidates

The use of animal-adapted strains of CoVs is critical for advanced MCM development as well and provides significant advantages over the use of alternative model systems. Though transgenic animals and naturally susceptible hosts can be used to demonstrate that MCMs diminish viral replication, an important proof of concept for early stage MCMs, animal-adapted strains that replicate human disease pathology provide a much more robust system for demonstrating the safety and efficacy of MCM candidates. Because adapted strains provoke a response from the host immune system, use of these strains can reveal MCM side effects or adverse reactions that are not seen in asymptomatic models, an important aspect of safety testing.

9.3.5.4.2 Vaccine Development Benefit 4: Developing Broad-Spectrum Vaccines

Chimeric bat-SARS CoV strains created using GoF approaches that adapt a virus to a new host are **uniquely capable** of providing reliable information about the broad-spectrum potential of CoV MCMs. Because most bat CoV strains cannot be cultured, the use of wild type viruses cannot provide information about whether CoV MCMs are capable of targeting a variety of SARS/MERS-like CoVs in addition to SARS and MERS. While expressing CoV Spike proteins in the context of other viruses (i.e., pseudotyped viruses and other chimeric virus systems) may be useful for screening MCM candidates targeting the Spike protein, all results must be confirmed using wild type strains (or CoV chimeric strains) due to significant differences in the behavior of chimeric viruses versus CoVs.

9.4 Introduction to GoF Research Involving Influenza Viruses

9.4.1 Overview of the Landscape of GoF Research Involving Influenza Viruses

Section 9.4 through Section 9.11 describe the benefits of GoF approaches involving influenza viruses, based on an analysis of the outcomes of GoF experiments published in the scientific literature. Our review of the influenza virus literature included the following virus strains:

- Human seasonal strains: currently circulating and historical influenza A H1N1 and H3N2 viruses and influenza B viruses,
- Human pandemic strains: the 1918 H1N1, 1957 H2N2, 1968 H3N2, and 2009 H1N1 viruses,

- Swine-origin strains: H3N2v and others, and
- Avian-origin strains: H5N1, H7N9, H9N2 and others.

We identified approaches involving influenza viruses that are reasonably anticipated to lead to the following phenotypic changes:

- Enhanced pathogen production as a result of changes in the replication cycle or growth,
- Enhanced morbidity and mortality in appropriate animal models,
- Altered host range,
- Enhanced transmission in mammals,
- Evasion of existing natural or induced immunity,
- Evasion of therapeutics, and
- Evasion of vaccines in development.

Through this document, the term "therapeutics" includes drugs that directly target viruses (e.g., influenza neuraminidase inhibitors), monoclonal antibody-based therapeutics, host immune modulators, and any other type of antiviral therapeutic. Influenza research that is reasonably anticipated to lead to evasion of diagnostics was not identified.

Descriptions of individual experimental approaches are provided within individual GoF phenotype sections. Of note, passaging of influenza viruses and coronaviruses in cells is essential for any experimental work involving live viruses, both to prepare virus stocks for experimental use and to conduct infection experiments. This applies to alt-GoF approaches, such as characterization of wild type viruses, as well as to GoF approaches. Because of the high mutation rates of RNA viruses, including influenza viruses and coronaviruses, such passaging inevitably selects for higher-yield viruses.[524] However, within the "enhanced virus production" phenotypic category, this analysis is restricted to those approaches that deliberately seek to enhance virus production through serial passaging, targeted genetic modification, or other approaches.

9.4.2 Use of Attenuated Strains of Influenza Viruses

Throughout the field of influenza research, the use of reassortant strains comprised of gene segments from a wild type strain and an attenuated, high-yield lab-adapted strain (e.g., A/Puerto Rico 8—PR8) is common. As described in Section 4.4.2.1, these strains are comprised of the HA and NA genes from a wild type strain and the remaining six genes from PR8 ("6:2R strains") and can be generated through reverse genetics or classical co-infection methods. 6:2R strains can be considered GoF strains by two criteria: (1) 6:2R strains exhibit enhanced virus production relative to the parental wild type strain (albeit reduced virus production relative to the parental lab-adapted strain), and (2) 6:2R strains exhibit enhanced pathogenicity relative to the parental lab-adapted strain (albeit reduced pathogenicity relative to the parental wild type strain). These strains are used for two purposes. In the context of vaccine production and basic science research that aims to elucidate mechanisms regulating the growth of vaccine viruses (Section 4.4.2.1), 6:2R strains are utilized due to their enhanced growth phenotype, which is desirable for efficient vaccine production. Therefore, when considering enhanced viral growth, the generation and use of 6:2R strains is considered to be a GoF approach. In contrast, in the context of research involving the study of other GoF phenotypes associated with the HA and NA proteins, 6:2R strains are utilized due to their attenuated phenotype, as a risk mitigation strategy. (In this context, 7:1R strains, comprised of the

[524] Parvin JD *et al* (1986b) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. *Journal of virology* 59: 377-383

HA and/or NA genes from the wild type strain and the remaining six or seven genes from PR8, may also be used.) Specifically, researchers may perform GoF approaches, such as serial passaging of viruses in the presence of cognate antibodies to select for antibody escape mutants, using 6:2R strains in lieu of wild type viruses. Thus, in these studies, 6:2R strains are subjected to an additional GoF approach. These studies include:

- Antigenic escape studies, which may lead to the generation of viruses that evade existing natural or induced adaptive immunity,
- Emergence of antiviral resistance studies, which may lead to the generation of viruses that evade therapeutics,
- Some approaches that may lead to the generation of viruses with enhanced pathogenicity, and
- Some approaches that may lead to the generation of viruses with altered host range in mammals and/or enhanced transmissibility in mammals.

In each of these sections, the use of 6:2R strains in place of wild type strains is considered to be an alternative approach for several reasons: (1) 6:2R strains are utilized due to their *attenuated* phenotype relative to parental, wild type strains, (2) the use of attenuated reassortant strains has been described as an alternative approach in the GoF debate, and (3) for a given GoF approach, the utility and limitations associated with the use of attenuated reassortant strains are different from those associated with the use of wild type strains and must be evaluated separately.

Several other risk mitigation strategies that involve the use of attenuated or replication-incompetent strains may be used for select GoF approaches in lieu of wild type strains. For those GoF approaches that may enhance pathogenicity, alter host range, or enhance transmissibility and that focus on the function of influenza proteins other than the HA and NA, another type of risk mediation reassortant may be used. Specifically, these reassortants comprise the HA and/or NA genes from a human seasonal flu strain, to which the population has pre-existing immunity, and up to the remaining six or seven genes from animal influenza strains or the 1918 H1N1 pandemic strain. As for attenuated reassortants with PR8, the use of these strains is considered to be an alternative approach because the purpose for their use is their attenuated phenotype and because their utility and limitations for a given GoF approach are different from those of the wild type strains.

For select GoF studies involving highly pathogenic avian influenza (HPAI) viruses, the multi-basic cleavage site (MBCS) of the viral surface glycoprotein HA, a major determinant of virulence, can be removed through deletion or mutation to mitigate risk. The MBCS is thought to mediate systemic replication and enhanced virulence in part by defining sensitivity to tissue specific proteases that are required for activation of the HA protein during infection. As a result, HPAIΔMBCS strains do not efficiently infect animal models and so cannot be used for *in vivo* studies; these attenuated strains can be used for select *in vitro* studies when cell culture media is supplemented with the appropriate proteases.

Replication-incompetent viruses can be used for the *in vitro* study of phenotypes underlying pathogenicity. In these model systems, viral replication and immune evasion pathways, both of which contribute to pathogenicity *in vivo*, can be assessed in cell culture lines that are engineered to stably express an essential viral protein that is missing from the "replication-incompetent" virus strains used for infection. For example, the replacement of the PB2 gene with a GFP-expression construct that has the necessary flanking, non-coding, and packaging sequences from the viral genome can only replicate in cell

lines that stably express exogenous PB2.[525] The result is a virus that is biologically constrained to replication in that cell line. Several replication incompetent model systems have been developed for the study of seasonal, pandemic, and animal influenza virus gene segments.

A final risk mitigation strategy that also modulates the replicative capacity of influenza viruses involves engineering binding sites for endogenous microRNAs (miRNAs) into the influenza virus genome. In cells that are expressing sufficient levels of the miRNA, miRNA binding to the viral RNA restricts viral replication. Incorporating target sites for miRNAs that are expressed in humans but not in an animal model of interest (e.g., ferrets) leads to the generation of a virus that is replication-competent in experimental animals but not humans, thus achieving "molecular biocontainment" of the virus. Langlois et al. pioneered this novel attenuation strategy in late 2013.[526] The authors incorporated target sites for miR-192, which is expressed in humans and mice but not ferrets, into the HA genome segment of two different strains: an attenuated lab reassortant strain and a seasonal H3N2 strain. The engineered strains displayed normal replication in ferrets but markedly reduced replication in mice and human cells, thereby providing proof of concept of this molecular biocontainment strategy. Importantly, incorporating miR-192 binding sites into the H3N2 strain did not prevent contact or airborne transmission of the virus in ferrets, demonstrating that the engineered virus behaves similarly to the wild type virus with respect to transmission dynamics. Though considered a promising risk mitigation approach by the influenza research community, other properties of the engineered viruses have not yet been extensively characterized, and the method has not been validated in other strains or using other miRNA target sites.

9.4.3 Organization of the Assessment of the Benefits of GoF Research Involving Influenza Viruses

The following chapters (9.5 through 9.11) summarize the results of the assessment of the benefits of GoF research involving influenza viruses. A more detailed analysis to further support the findings described in these chapters is presented in Appendix IV Section 15.2 through Section 15.8. As the relative ability of a given GoF (or alt-GoF) approach to address a particular scientific knowledge or public health gap often hinges on nuanced differences between the benefits and limitations of different approaches, readers who seek an in-depth understanding of the benefits of GoF research are directed to Appendix IV Section 15.

9.5 Influenza viruses: Benefits of GoF Research that Enhances Virus Production

9.5.1 Summary

This section describes the benefits of GoF research that is reasonably anticipated to lead to enhanced production of influenza viruses as the result of changes in the replication cycle or growth. Such GoF studies were found to generate scientific knowledge, have direct applications in vaccine development, and are likely to have economic benefits. Alt-GoF approaches that may generate similar benefits were also identified and analyzed. At present, GoF studies resulting in enhanced influenza production have unique and direct benefits, particularly to vaccine development and production. Chapter 9.5 provides an overview of these benefits, including basic background and Supporting Information; a fully referenced and more thorough discussion of these benefits can be found in Appendix IV Section 15.2.

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[525] Ozawa M *et al* (2011) Replication-incompetent influenza A viruses that stably express a foreign gene. *The Journal of general virology* 92: 2879-2888

[526] Langlois RA *et al* (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844-847

9.5.1.1 Benefits of GoF that Enhances Virus Production to Scientific Knowledge

- GoF approaches are:
  - Uniquely capable of discovering mutations that enhance the growth of vaccine viruses to greater-than-wildtype levels, which is desirable for vaccine production and provides a foundation for understanding the mechanistic basis of high growth of vaccine viruses.
  - Uniquely capable of demonstrating that particular markers are necessary and sufficient to enhance the growth of vaccine viruses, and
  - Uniquely critical for generating information that can be translated to the vaccine production process.
- Alternative experimental approaches are:
  - Limited to the study of genetic markers and phenotypes underlying naturally high levels of growth, and
  - Limited to confirming that mutations are necessary for high growth but cannot be used to demonstrate that mutations enhance growth, and thus cannot provide information that can be applied to the vaccine production process.

9.5.1.2 Benefits of GoF that Enhances Virus Production to Vaccine Production

- GoF approaches are:
  - Uniquely critical for the *current* capability to produce sufficient and effective vaccines for seasonal and pandemic influenza,
  - Capable of improving the quality and availability of influenza vaccines in the *future* by:
    - Shortening production timelines for egg- and cell-based vaccines, which translates to faster vaccine availability during a pandemic and improved vaccine match for seasonal influenza vaccines by enabling strain selection closer to the start of flu season,
    - Enabling the production of well-matched vaccines for strains that mutate to alter their antigenicity during growth in eggs or cells, which is a unique benefit of GoF approaches.
  - Because there are likely to be no regulatory barriers for incorporating genetic markers identified through GoF research into vaccine strains, this benefit can be achieved in the immediate to near future.
- Alternative approaches are:
  - Incapable of replacing current vaccine production processes in the near-term,
  - Capable of improving the quality and availability of influenza vaccines in the *future* through several different mechanisms:
    - Incorporating adjuvants into existing vaccines would shorten production timelines by enabling the use of smaller quantities of antigen per vaccine dose. However, no adjuvanted seasonal vaccines are FDA-licensed, and the development and licensing procedures for new adjuvanted vaccines are lengthy and expensive,
    - New virus-free vaccine platforms that do not rely on GoF are capable of producing strain-specific vaccines on shorter timescales than existing egg- and cell-based production

systems. However, only one recombinant vaccine is licensed, and the development and licensing procedures for new vaccines are lengthy and expensive.

- Developing a universal or broad-spectrum flu vaccine would obviate the need for annual production of seasonal vaccines and for production of strain-specific vaccines in response to the emergence of a novel pandemic strain. However, the feasibility of producing a universal flu vaccine is unknown.
- Developing pre-pandemic vaccines would lead to faster vaccine availability during a pandemic. However, because resources for pre-pandemic vaccine development and stockpiling are limited, pre-pandemic vaccines function to bridge the gap between the emergence of a novel strain and the large-scale deployment of strain-specific vaccines.
- Improving strain selection capabilities will reduce the likelihood of vaccine mismatch due to incorrect prediction of which strains will be circulating six to nine months hence. The realization of this benefit, which complements efforts to shorten vaccine production timelines by addressing a different shortcoming in the existing vaccine production process, depends on scientific advancements.

9.5.1.3 Economic Benefits of GoF that Enhances Virus Production

- Increasing the yields of vaccine viruses, using information or products derived from GoF approaches that enhance virus production, is likely to lower the cost per vaccine dose by enabling the production of a greater number of vaccine doses using the same materials.

GoF approaches that enhance virus production do not benefit surveillance, informing policy decisions, or the development of therapeutics or diagnostics.

9.5.2 Overview of GoF Research Landscape: Enhanced Virus Production

9.5.2.1 Generation of Attenuated, High-Yield Candidate Vaccine Viruses Through Reassortment

Reassortment between a wild type strain and an attenuated, high-yield vaccine backbone strain generates a "Candidate Vaccine Virus" (CVV), which comprises the HA and NA genes from the wild type strain and the remaining six "internal genes" from the vaccine backbone strain. CVVs are attenuated and exhibit higher levels of growth relative to the parental, wild type virus. CVVs may be generated through classical reassortment methods, which involve co-infection of eggs or cells with the wild type strain and the vaccine backbone strain followed by antibody-based selection for viruses with the correct surface antigens, or through reverse genetics.[527] CVVs serve as the basis of vaccine strains that are used for the production of influenza vaccines in eggs or cells. Additionally, in the context of academic research, comparing the sequences of CVVs with varied growth properties enables the identification of mutations that are associated with high yield.

9.5.2.2 Serial Passaging of Viruses in Eggs or Cells

Serial passaging of viruses in eggs or cells selects for higher-yield viruses. This approach is currently used for the production of influenza vaccines in eggs or cells as well as for basic science research on the

[527] Use of classical reassortment methods to generate CVVs may lead to the generation of a 5:3 reassortment strain which includes the HA, NA, and one additional gene from the wild type strain and the remaining five genes from the vaccine backbone strain.

mechanisms underlying high growth of influenza viruses. For vaccine production, manufacturers serially passage CVVs in eggs or cells to generate high-yield vaccine seed strains that can be used for large-scale production of vaccines. In the context of scientific research, serial passaging of viruses in eggs or cells followed by sequencing of the emergent higher-yield viruses enables the identification of mutations that are sufficient to enhance the growth of viruses. Subsequently, mutant viruses are subjected to antigenic characterization using the hemagglutinin inhibition (HAI) assay or other assays to identify which mutations confer high growth without changing the antigenicity of the strain. For research purposes, this approach is most commonly carried out using vaccine backbone strains and CVVs but may also be carried out using wild type strains.

9.5.2.3 Forward Genetic Screen to Identify Mutations that Confer High Growth to Viruses

Forward genetic screens, which involve random mutagenesis of viruses followed by limited passaging to select for mutants with high growth properties, enable the identification of mutations that confer high growth to viruses. Forward genetic screens involving vaccine backbone strains and CVVs lead to the identification of mutations that are sufficient to enhance the yields of vaccine viruses. Subsequently, mutant viruses are subjected to antigenic characterization using the hemagglutinin inhibition (HAI) assay or other assays to determine which mutations confer high growth without altering the antigenicity of the strain.

9.5.2.4 Targeted Mutagenesis of Viruses to Introduce Mutations That are Associated with High Growth

Targeted mutagenesis of viruses to introduce mutations that are associated with high growth, followed by characterization of virus yields relative to the parental virus, demonstrates that a mutation or set of mutations is necessary and sufficient to confer high growth. Subsequently, antigenic characterization assays are performed to confirm that the mutations have not altered the antigenicity of the virus, and the mutant strain is subjected to several rounds of passaging in eggs or cells to ensure that it is genetically stable – that is, that it does not acquire additional mutations that alter its antigenicity upon further growth. This knowledge provides a foundation for follow-up studies investigating the mechanistic basis of the high-growth phenotype (e.g., the use of cell biological assays, biochemical assays, and other assays to explore how the mutation enhances growth). Notably, these mutations may have been discovered through a GoF approach, such as serial passaging or a forward genetic screen, or through an alt-GoF approach, such as comparative analysis of wild type sequences.

Finally, it should be noted that experimental approaches involving targeted genetic modification of the viral polymerase complex of avian viruses to render it more "human-like" (through site-directed mutagenesis or reassortment between human and avian viruses) is also likely to enhance virus replication. However, as the primary goal of those studies is to gain insight into the mechanisms underlying adaptation of avian viruses to mammals, those studies are discussed in the "enhanced transmission in mammals" section.

9.5.3 Identification of Potential Benefits and Limitations of GoF Approaches

In this section, the potential benefits of GoF research that enhances virus production in each benefit category listed in the NSABB Framework are discussed.

9.5.3.1 Scientific Knowledge Benefits

The mechanistic basis of high growth of vaccine viruses in eggs or cells is not well understood. Current strategies for producing vaccine viruses do not consistently produce high-yield strains, which are needed

for efficient vaccine production. In addition, further boosting the growth properties of all vaccine strains has potential to increase the efficiency of the existing vaccine production process. The genetic and phenotypic traits that promote the growth of vaccine strains are largely unknown.

GoF approaches that enhance virus production have the potential to address this scientific knowledge gap by providing insight into the genetic and mechanistic basis of the enhanced growth phenotype. Specifically, serial passaging of viruses in eggs/cells and forward genetic screens followed by selection of high-growth mutants enable the identification of mutations that are sufficient to confer higher-than-wildtype levels of growth to any virus strain, though results may not translate to other virus strains. Comparative analysis of the sequences of CVVs with varying growth properties can also lead to the identification of mutations that are associated with naturally high levels of growth and may be more likely to uncover determinants of high growth that are conserved across multiple strains. However, comparative sequence analysis is unlikely to uncover genetic markers associated with greater-than-wild type levels of growth because it is limited to analysis of existing isolates. Finally, targeted mutagenesis can be used to demonstrate that a particular mutation or set of mutations is necessary and sufficient to enhance the growth of a vaccine virus strain, across multiple strain contexts. Collectively, these approaches provides a foundation for follow-up structural, biochemical, and cell biological assays investigating the phenotypic consequences of the mutations to gain insight into the mechanisms underlying the enhanced growth phenotype.

9.5.3.2 Surveillance

All other GoF approaches are focused on identifying mutations that confer high growth to vaccine viruses (either candidate vaccine viruses or vaccine backbone strains). Because these viruses have no correlate in nature, this information does not inform the interpretation of genetic surveillance data from animals or humans.

9.5.3.3 Development and Production of Vaccines

9.5.3.3.1 Background – Shortcomings in Existing Influenza Vaccine Production Processes

GoF approaches that enhance virus production have the potential to benefit the development of influenza vaccines, which are strain-specific. Due to antigenic drift of circulating seasonal influenza viruses, the strain composition of influenza vaccines must be updated annually, and the CDC's Advisory Committee on Immunization Practices recommends annual influenza vaccination for all people ages six months and older.[528] Currently, over 99% of seasonal influenza vaccines used in the US are produced in eggs or cells, and the same systems and facilities would be used to produce pandemic vaccine in response to the emergence of a novel pandemic strain of influenza.[529,530] For production of both seasonal and pandemic influenza vaccines, the vaccine production cycle spans six to eight months.[531,532]

Though the influenza vaccine development and production systems are well-established, interviews with stakeholders in the influenza research and public health communities highlighted that the lengthy production timelines for existing egg- and cell-based vaccines critically limit the mitigating impact of

[528] CDC's Advisory Committee on Immunization Practices (ACIP) Recommends Universal Annual Influenza Vaccination. http://www.cdc.gov/media/pressrel/2010/r100224.htm. Last Update Accessed September 15, 2015.

[529] Dowling B. Protein Sciences' N.Y. Factory Licensed For Flu Vaccine Production. http://www.courant.com/business/hc-protein-sciences-pearl-river-approval-20150513-story.html. Last Update 13 May 2015. Accessed 14 September 2015.

[530] CDC. What You Should Know for the 2015-2016 Influenza Season. http://www.cdc.gov/flu/about/season/flu-season-2015-2016.htm. Last Update Accessed September 15, 2015.

[531] (2015c) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

[532] Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*. 2nd Edition edn, pp 352-370.

influenza vaccination on the morbidity and mortality associated with influenza outbreaks. Lengthy vaccine production timelines impact the quality and availability of seasonal and pandemic flu vaccines differently. In the context of seasonal flu epidemics, existing production timelines necessitate strain selection nine months in advance of the peak of the target flu season.[533] As a result, one or more vaccine strains are often imperfectly matched to circulating strains, which reduces the efficacy of the vaccine.[534] In the context of pandemics, vaccines are simply unavailable to protect the public until at least six months into the outbreak.[535,536]

9.5.3.3.2 Potential Benefits and Limitations of GoF Approaches to Current Influenza Vaccine Production

GoF approaches are core aspects of the current process for producing influenza vaccines. To be suitable for large-scale manufacturing of vaccine virus, a selected field isolate must be attenuated and its growth in eggs/cells must be enhanced. This enhancement is achieved through the use of two different GoF approaches: (1) a CVV is created through reassortment between the field isolate and an attenuated, high-yield vaccine backbone strain and (2) the CVV is serially passaged in eggs or cells to increase its yield. Collectively, these GoF approaches increase HA antigen yield by yield at least 12-fold relative to the cognate wildtype strain.[537] (It should be noted that manufacturers report production increases in terms of HA antigen yield rather than viral titer because the FDA requires that a certain quantity of HA antigen be present in each vaccine dose. Increases in viral titer correlate with increases in HA antigen yields.)[538,539] The use of high-growth reassortant viruses generated through GoF methods enables the production of over 170 million doses of seasonal influenza vaccine annually and would enable the production of a similar number of doses of pandemic vaccine six to eight months after the emergence of a novel pandemic strain.[540]

9.5.3.3.3 Potential Benefits and Limitations of GoF Approaches to Future Influenza Vaccine Production

The insights gleaned from GoF approaches that enhance virus production also have the potential to improve vaccine production practices in the future through two distinct mechanisms: (1) shortening vaccine production timelines, and (2) improving the match between the virus strains used as the basis of vaccine strains and the strains that are circulating during flu season (referred to as "vaccine match").

First, GoF approaches have the potential to shorten vaccine production timelines by increasing the yields of vaccine viruses, which govern the rate at which vaccines are produced and thus serves as a key determinant of the time needed for egg- and cell-based vaccine production. GoF approaches can increase CVV yields in two ways: (1) through the direct use of higher-yield CVVs and (2) through the incorporation of genetic markers that confer high growth into existing CVVs using targeted mutagenesis. Shortening vaccine production timelines improves seasonal and pandemic influenza vaccines through different mechanisms. In the context of pandemics, faster vaccine production translates to vaccine availability earlier during the pandemic. In the context of seasonal flu epidemics, the ability to produce

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533 Ibid.
534 (2015n) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.
535 Borse RH *et al* (2013) Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. *Emerging infectious diseases* 19: 439-448
536 (2015e) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.
537 Ibid.
538 Food and Drug Administration. Annex 5. Vaccination Development and Production - Draft. http://www.hsdl.org/?view&did=459937. Last Update Accessed September 15, 2015.
539 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.
540 CDC. What You Should Know for the 2015-2016 Influenza Season. http://www.cdc.gov/flu/about/season/flu-season-2015-2016.htm. Last Update Accessed September 15, 2015.

vaccines in a shorter period of time enables strain selection closer to the start of flu season, increasing the likelihood that the vaccine strains will match the circulating strains during peak flu season. Ultimately, increasing the availability of vaccines during a pandemic and increasing the efficacy of vaccines during seasonal flu epidemics will reduce human morbidity and save lives.[541]

One key constraint on the benefits afforded by improvements to CVV yields is the limited production capacity of eggs and cells. Current *egg*-based vaccine production systems are at or near maximal levels of production, suggesting that the benefits of GoF research are largely limited to improving the growth of "poor" CVVs.[542] However, because many CVVs based on zoonotic viruses and seasonal H3N2 viruses grow poorly in eggs, simply improving their production would significantly benefit public health.[543,544] In contrast, the production capacities of *cell*-based systems have not yet plateaued, thus GoF research that improves CVV yields has the potential to benefit production of vaccines for all influenza sub-types using cell-based systems.[545] Importantly, because minor modifications to existing CVVs are unlikely to require FDA approval for use in vaccine production, these benefits can be realized in the immediate future.[546]

Second, the insights derived from GoF research can improve vaccine match for vaccines based on strains that tend to mutate upon growth in eggs or cells, which may lead to antigenic changes and poor vaccine match. In particular, H3N2 strains often acquire antigenicity-altering mutations upon growth in eggs, which is especially concerning given that H3N2 strains tend to cause more severe disease than H1N1 strains.[547,548,549,550] Mutations that enhance the growth of these strains without altering antigenicity, identified through GoF studies, can be incorporated into CVVs to enable the production of vaccines that match the antigenicity of selected strains.

9.5.3.4 Therapeutics and Diagnostics

Information about mutations that confer high growth to vaccine viruses or about mutations that rescue the growth of antiviral resistant strains is not relevant to the development of therapeutics.

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541 Borse RH *et al* (2013) Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. *Emerging infectious diseases* 19: 439-448

542 (2015e) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

543 (2015o) Candidate vaccine virus development. Interviews with Influenza Researchers Involved in Candidate Vaccine Virus Development.

544 (2015n) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

545 (2015e) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

546 Ibid.

547 (2015o) Candidate vaccine virus development. Interviews with Influenza Researchers Involved in Candidate Vaccine Virus Development.

548 Barman S *et al* (2015) Egg-adaptive mutations in H3N2v vaccine virus enhance egg-based production without loss of antigenicity or immunogenicity. *Vaccine* 33: 3186-3192

549 Huang SSH *et al* (2011) Comparative Analyses of Pandemic H1N1 and Seasonal H1N1, H3N2, and Influenza B Infections Depict Distinct Clinical Pictures in Ferrets. *PLoS ONE* 6: e27512

550 Kaji M *et al* (2003) Differences in clinical features between influenza A H1N1, A H3N2, and B in adult patients. *Respirology (Carlton, Vic)* 8: 231-233

The process of developing influenza diagnostics is well-established [551,552] GoF research that leads to the identification of genetic markers that confer GoF phenotypes, including enhanced virus production, does not inform diagnostic development.

9.5.3.5 Informing Policy Decisions

Similarly, information about mutations that confer high growth to vaccine viruses does not inform the analysis of genetic surveillance data, so this information does not benefit policy decisions about public health preparedness.

9.5.3.6 Economic Benefits

Increasing the yields of vaccine viruses, using information or products derived from GoF approaches that enhance virus production, is likely to lower the cost per vaccine dose by enabling the production of a greater number of vaccine doses using the same materials. However, the economic benefits of enhancements to vaccine virus yields to vaccine production were not explored in detail in this report.

9.5.4 Identification of the Potential Benefits and Limitations of Alt-GoF That Provide Similar Potential Benefits to the GoF Being Examined

In this section, an overview of alternative (alt-GoF) approaches that yield the same or similar benefits as the GoF approaches described above is provided. Two types of alt-GoF approaches are reviewed: (1) alternative experimental approaches that can provide the same or similar scientific information as GoF experimental approaches, and (2) alternative scientific and technical innovations that can yield the same public health benefits as GoF approaches but through different mechanisms.

9.5.4.1 Potential Benefits and Limitations of alt-GoF Approaches to Scientific Knowledge

Several alternative experimental approaches can provide insight into the genetic and mechanistic basis of high growth of vaccine viruses, which complement GoF approaches that lead to the identification of genetic traits that enhance virus growth. First, sequence comparison of wildtype strains with varied growth properties can lead to the identification of mutations that are associated with naturally high levels of growth. Of note, because of the importance of genetic context on multi-genic traits such as fitness, mutations that confer high growth to wildtype strains may not confer high growth to vaccine strains (i.e., reassortants that include the HA and NA from the field isolate and the remaining six genes from a vaccine backbone strain). Additionally, this approach depends on the existence of high-growth strains in nature and cannot identify mutations that confer exceptional yields.

Genetic screens to identify mutations that reduce growth (i.e., Loss of Function, or LoF) can lead to the discovery of mutations that are *necessary* for growth. A major limitation of this approach is that it may uncover mutations that reduce growth for "trivial," reasons (i.e., that modulate critical aspects of virus function that are necessary for viability but do not directly contribute to high growth). An additional drawback is that it is much less efficient than its GoF counterpart because mutants must be screened for reduced growth (versus selection for high growth through passaging). Finally, the utility of the information gleaned from LoF screens also depends on the existence of high-growth strains in nature.

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551 New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

552 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

LoF approaches may also be used to confirm that a particular amino acid residue (discovered through GoF or alt-GoF approaches) is necessary for high growth. However, the marker may not be sufficient to enhance growth if introduced into a different strain, limiting the utility of this result for vaccine production.

9.5.4.2 Potential Benefits and Limitations of alt-GoF Approaches to Vaccine Production

9.5.4.2.1 Potential Benefits and Limitations of Alt-GoF Approaches to Current Influenza Vaccine Production

High-yield, attenuated vaccine viruses generated through GoF approaches are currently used for production of egg- and cell-based vaccines. The use of strains with wild type growth properties represents one alternative to the use of high-yield vaccine viruses. This could involve the direct use of wild type strains or the use of novel reassortant strains that are attenuated but exhibit wild type levels of virus production. Because most influenza strains grow poorly in eggs/cells, the concentration of virus antigen in eggs/cells infected with strains with wild type growth properties would be so low that existing manufacturing processes would likely fail to purify antigen that meets FDA standards, resulting in **no vaccine produced**. Alternatively, a wild type isolate with exceptional growth properties could be used to produce the same number of doses over a longer period of time or to produce a smaller number of doses over the same period of time. For example, use of a wild type isolate the grows four times as well as an average strain would either lengthen the vaccine production timeline to more than one year or would reduce the number of doses produced two- to three-fold. Additionally, because wild type viruses with exceptional yields *and* appropriate antigenic properties are unlikely to be available, this scenario would likely result in the use of a poorly matched strain, leading to the production of a less effective vaccine.

Additionally, neither alternative (i.e., use of wild type strains or use of novel reassortants with wild type growth properties) can be implemented immediately. Large-scale production using wild type isolates for the purpose of producing inactivated vaccines would pose significant risks to vaccine manufacturers prior to the inactivation step, presumably requiring the construction of new manufacturing facilities capable of virus production under higher biocontainment conditions. Of note, field isolates cannot be used as a basis for live vaccines due to their pathogenicity. The alternative, use of attenuated vaccine viruses with wild type growth properties, would necessitate the development, and perhaps subsequent FDA licensing, of novel vaccine backbone strains that attenuate but do not confer high growth to reassortant viruses.

As described above, production of virus-based vaccines in eggs/cells necessitates passaging of the antigenic strain of interest to produce enough stock virus to infect eggs/cells for large-scale manufacturing, which inevitably selects for higher-yield viruses due to the high mutation rate of influenza viruses.[553] If this passaging were considered to be a GoF approach, in addition to the approaches described above that deliberately enhance the yields of vaccine viruses, then completely avoiding manipulations that are reasonably expected to enhance virus production precludes production of egg- and cell-based influenza vaccines. In that case, virus-free vaccine platforms, such as recombinant or DNA-based vaccines, represent an alternative to egg- and cell-based flu vaccines.[554,555,556] However, only one recombinant flu vaccine is commercially available and is only approved for use in people 18 years of age and older. This vaccine represented just 50,000 of more than 140 million doses administered during the

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553 Parvin JD *et al* (1986b) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. *Journal of virology* 59: 377-383

554 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

555 Kim JH, Jacob J (2009) DNA vaccines against influenza viruses. *Current topics in microbiology and immunology* 333: 197-210

556 Bright R. Review of New Vaccine Platforms and Influenza Vaccine Pipeline. http://www.who.int/influenza_vaccines_plan/resources/bright.pdf. Last Update Accessed September 15, 2015.

2014 – 2015 flu season.[557,558] Although other recombinant vaccines are in late stages of development, given the long and expensive product development cycle for new influenza vaccines– spanning eight to 12 years and costing 300 million to one billion dollars including research, clinical development, and registration with the FDA– alternative, virus-free flu vaccine platforms are not a viable *replacement* for egg- and cell-based vaccines in the immediate future.[559]

9.5.4.2.2 Potential Benefits and Limitations of Alt-GoF Approaches to Future Influenza Vaccine Production

Several alternative scientific and technical innovations have the potential to benefit vaccine production in the future. Of note, some of these innovations can improve the production of both seasonal and pandemic influenza vaccines, whereas others can only improve production of seasonal or pandemic vaccines. These differences reflect the fact that seasonal flu vaccines are produced annually in advance of flu season, whereas pandemic vaccines are produced in response to the emergence of a novel pandemic strain.

An alternative approach for improving vaccine virus yields without enhancing the inherent growth properties of CVVs is through modulation of the host cells that are used to produce virus. Specifically, identification of host genes that suppress viral growth provides a basis for development of specialized knockout cell lines that permit higher virus yields.[560] This approach has potential to benefit the production of seasonal and pandemic flu vaccines but has been tested on a limited number of strains. No modified cell lines are currently FDA-approved for vaccine production, and only one cell-based vaccine that could potentially make use of this technology is licensed in the US.[561] Cell lines must undergo extensive testing in order to be FDA-approved for influenza vaccine production prior to their commercial use, which will delay realization of this benefit.[562,563] Finally, because this approach increases viral titer, it is not less risky than GoF approaches than enhance the growth properties of vaccine viruses.

An adjuvant is a substance that is added to a vaccine to boost the body's immune response to the vaccine, and including an adjuvant in a vaccine may enable the use of a smaller quantity of antigen to induce the same level of protection ("dose sparing"). [564] Thus, incorporating adjuvants into existing egg- and cell-based vaccines represents a different strategy for shortening production timelines, by enabling production of the same number of doses over a shorter period of time. Most licensed vaccines in the US are not adjuvanted – one seasonal vaccine containing adjuvants was recently approved for use in people aged 65

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557 Dowling B. Protein Sciences' N.Y. Factory Licensed For Flu Vaccine Production. http://www.courant.com/business/hc-protein-sciences-pearl-river-approval-20150513-story.html. Last Update 13 May 2015. Accessed 14 September 2015.

558 Protein Sciences. Flublok. http://www.proteinsciences.com/FVAC.htm. Last Update Accessed September 15, 2015.

559 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

560 Hamamoto I *et al* (2013) High yield production of influenza virus in Madin Darby canine kidney (MDCK) cells with stable knockdown of IRF7. *PloS one* 8: e59892

561 TABLE. Influenza vaccines — United States, 2015–16 influenza season. http://www.cdc.gov/flu/protect/vaccine/vaccines.htm. Last Update Accessed September 14, 2015.

562 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

563 FDA. Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications http://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm202439.pdf. Last Update Accessed September 15, 2015.

564 CDC. Vaccine Adjuvants. http://www.cdc.gov/vaccinesafety/concerns/adjuvants.html. Last Update Accessed September 15, 2015.

and older, and one licensed pandemic influenza vaccine contains adjuvants.[565,566,567,568] Nonetheless, use of adjuvants to improve the immunogenicity of seasonal influenza vaccines is an active area of research. The major barrier to realization of this benefit is that existing vaccines that are re-formulated with adjuvant are considered new drugs by the FDA and as such must undergo the standard licensure pathway for unadjuvanted vaccines, which will delay their widespread availability due to the time needed to generate the needed safety and efficacy data.[569,570,571] This approach has potential to benefit the production of seasonal and pandemic flu vaccines.

Developing new vaccine platforms with faster production timelines represents a third alternative approach for shortening the time needed for production of strain-specific vaccines. Recombinant vaccines, which are virus-free vaccines comprised of recombinant influenza proteins produced in insect cells or other protein expression systems such as plants, represent the most developed and promising approach.[572,573] Although only one recombinant vaccine is currently FDA-licensed, several other recombinant vaccines are in late stages of development, and experts in the influenza vaccine field expect the production and use of this type of vaccine to increase over the next several decades.[574,575] However, as mentioned above, the time needed for completion of clinical trials and licensing delays the ability of this technology to impact influenza vaccination systems in the US in the near term (i.e., within the next few years).[576] Virus-free vaccine platforms can be used for the production of seasonal and pandemic influenza vaccines.

A universal or broad-spectrum flu vaccine would obviate the need for yearly production of strain-specific vaccines as well as the need to produce a strain-specific vaccine in response to the emergence of a novel pandemic strain. Such a vaccine could be administered in advance of a pandemic, generating pre-existing immunity in the population, or could be stockpiled and immediately deployed following the start of a pandemic. However, universal or broader-spectrum vaccines are still in early stages of development and represent an extremely challenging prospect given the high mutability of influenza viruses.

Development of pre-pandemic vaccines against circulating zoonotic influenza strains with pandemic potential would also lead to faster vaccine availability during a pandemic caused by a closely related

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565 Ibid.

566 Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm376289.htm. Last Update Accessed September 15, 2015.

567 FDA. FDA approves first seasonal influenza vaccine containing an adjuvant. FDA News Release. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm474295.htm. Last Update November 24, 2015. Accessed November 28, 2015.

568 Novartis. FLUAD® (MF59®-Adjuvanted Influenza Vaccine) Fact Sheet. https://www.novartis.com/sites/www.novartis.com/files/Fluad_Fact_Sheet.pdf. Last Update Accessed September 15, 2015.

569 Montomoli E *et al* (2011) Current adjuvants and new perspectives in vaccine formulation. *Expert Rev Vaccines* 10: 1053-1061

570 Food and Drug Administration. Vaccine Product Approval Process. http://www.fda.gov/BiologicsBloodVaccines/DevelopmentApprovalProcess/BiologicsLicenseApplicationsBLAProcess/ucm133096.htm. Last Update 24 August 2015. Accessed 14 September 2015.

571 Gruber M. Regulatory Pathways Supporting Development and Approval of Vaccines Formulated with Novel Adjuvant: Regulatory Considerations and Challenges. http://www.fda.gov/downloads/EmergencyPreparedness/MedicalCountermeasures/UCM292045.pdf. Last Update 2012. Accessed 14 September 2015.

572 Bright R. Review of New Vaccine Platforms and Influenza Vaccine Pipeline. http://www.who.int/influenza_vaccines_plan/resources/bright.pdf. Last Update Accessed September 15, 2015.

573 (2015e) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

574 TABLE. Influenza vaccines — United States, 2015–16 influenza season. http://www.cdc.gov/flu/protect/vaccine/vaccines.htm. Last Update Accessed September 14, 2015.

575 (2015e) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

576 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370

strain. Developing pre-pandemic CVVs and carrying out clinical trials would shorten vaccine production timelines, and stockpiling bulk antigen would allow for near-immediate deployment of vaccine following emergence of a pandemic strain. In addition, manufacturers' experience with production of the vaccine would likely streamline subsequent large-scale production during the pandemic.[577] Although the pre-pandemic vaccine strain is unlikely to exactly match the strain that emerges to cause a pandemic, use of adjuvants and prime-boost regimens broaden the protection that can be achieved using a strain-specific vaccine, such that pre-pandemic vaccines are highly likely to provide some level of protection against infection with a similar strain.[578,579,580,581,582] The benefit of developing pre-pandemic vaccines is constrained by the fact that resources for the development and stockpiling of pre-pandemic vaccines are limited. Resource limitations necessitate targeted, risk-based investments in pre-pandemic vaccine development, which are informed by GoF approaches that enhance the transmissibility and virulence of influenza viruses, discussed in detail in Section 9.6.3.3.[583]

Shortening vaccine production timelines (through GoF or alt-GoF approaches) represents one strategy for improving the match between seasonal flu vaccines and circulating strains, by enabling the selection of vaccine strains closer to the start of flu season. However, as long as vaccine strains must be selected in advance of flu season, there remains the possibility of vaccine mismatch due to incorrect prediction of which strains will be dominant during the target flu season. Therefore, improving strain selection capabilities represents a completely different mechanism for improving the efficacy of seasonal influenza vaccines. As discussed in detail in Section 9.8.5.3.1, both GoF approaches that lead to evasion of existing natural or induced adaptive immunity and alt-GoF approaches have potential to improve strain selection capabilities.

9.5.5 Comparison and analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

In this section, the potential benefits of GoF research that enhances virus production *relative* to alt-GoF approaches are discussed, in each benefit category that GoF approaches can address

9.5.5.1 Benefits to Scientific Knowledge

GoF approaches are **uniquely capable** of discovering mutations that enhance the growth of vaccine viruses to greater-than-wildtype levels, which is important for the efficient production of egg- and cell-based influenza vaccines. In addition, GoF approaches are **uniquely capable** of demonstrating that particular markers are necessary and sufficient to enhance the growth of vaccine viruses, which is essential for translation of information about high-growth markers to the vaccine production process. Together, this information provides a foundation for follow-up studies investigating the mechanistic basis of high virus yields. Alternative approaches have significant limitations for the study of high virus yields,

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577 (2015e) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

578 Ibid.

579 (2015d) Influenza Vaccines. Interviews with Public Health Professionals Involved in Preventing and Responding to Influenza Outbreaks.

580 Smith GE *et al* (2013) Development of influenza H7N9 virus like particle (VLP) vaccine: homologous A/Anhui/1/2013 (H7N9) protection and heterologous A/chicken/Jalisco/CPA1/2012 (H7N3) cross-protection in vaccinated mice challenged with H7N9 virus. *Vaccine* 31: 4305-4313

581 Middleton D *et al* (2009) Evaluation of vaccines for H5N1 influenza virus in ferrets reveals the potential for protective single-shot immunization. *Journal of virology* 83: 7770-7778

582 Khurana S *et al* (2010) Vaccines with MF59 adjuvant expand the antibody repertoire to target protective sites of pandemic avian H5N1 influenza virus. *Sci Transl Med* 2: 15ra15

583 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

relative to GoF approaches. Comparative sequence analysis of wildtype viruses is limited to the study of genetic markers and phenotypes underlying naturally high levels of growth, which provides a limited range of information relative to GoF approaches. LoF approaches are much less efficient than GoF approaches, and mutations that attenuate growth may not confer high growth if introduced into a new strain. Neither alternative approach is capable of generating information that can be applied to vaccine productions.

9.5.5.2 Benefits to Vaccine Production

9.5.5.2.1 Benefits to Current Production of Influenza Vaccines

GoF approaches to enhance the growth of attenuated vaccine strains are a **uniquely critical component** of the current ability to produce sufficient and effective vaccines for seasonal and pandemic influenza. The use of strains with field-like growth properties in lieu of high growth reassortants generated using GoF approaches would result in the production of no vaccines, the production of a lesser quantity of vaccines that poorly match circulating strains, or the production of vaccines that poorly match circulating strains over an extended time period. Furthermore, using field strains would require the construction of new manufacturing facilities capable of virus production under higher biocontainment conditions, and using attenuated strains with wildtype levels of growth would require the development and possible FDA licensure of new vaccine backbone strains that are attenuated but do not confer high growth to reassortant viruses, delaying the implementation of either alternative approach. Recombinant vaccines and other virus-free vaccine platforms represent a promising approach for future influenza vaccine production, but the one recombinant vaccine that is currently licensed represents less than 1% of seasonal influenza vaccines administered annually, and lengthy regulatory processes will delay the availability of additional virus-free vaccines in the future.

9.5.5.2.2 Benefits to Future Production of Pandemic Influenza Vaccines

Both GoF approaches to improve CVV yields and alternative approaches have potential to reduce the time lag between the emergence of a novel pandemic strain in human populations and the widespread availability of a vaccine, thus reducing human morbidity and mortality during an influenza pandemic. GoF approaches to improve the yields of vaccine viruses are **uniquely capable** of achieving this benefit in the immediate to near term because use of this information capitalizes on existing infrastructure and faces no regulatory barriers to translation. Adjuvanted vaccines and virus-free vaccines also have shorter production timelines than existing egg- and cell-based vaccines, but the widespread availability of both types of vaccines will be delayed due to time needed for vaccine development and the generation of safety and efficacy data that is required for FDA licensure of new flu vaccines. Universal flu vaccines are not a viable option for protection against pandemic influenza in the near future, and whether the development of a universal or broad-spectrum vaccine is possible is unknown. The development of pre-pandemic vaccines represents a promising strategy due to their ability to provide broad-spectrum protection when adjuvanted. However, because resources limit the scope of the USG's investment in pre-pandemic vaccines, these vaccines will serve to bridge the gap between the emergence of a novel strain and widespread availability of vaccines and must be complemented by innovations to shorten vaccine production timelines.

9.5.5.2.3 Benefits to Future Production of Seasonal Influenza Vaccines

Both GoF approaches and alt-GoF approaches have potential to improve the match between seasonal influenza vaccines and strains that are circulating during flu season, thus improving vaccine efficacy and decreasing human morbidity and mortality associated with seasonal flu epidemics. This benefit can be achieved through several different mechanisms. One strategy is to improve the production of strains that

mutate to alter their antigenicity upon growth in eggs or cells, which results in the production of vaccines that are poorly matched to the selected strains. GoF approaches are uniquely capable of generating high-yield, genetically stable CVVs that do not acquire antigenicity-altering mutations during passage in eggs or cells. A different strategy is to shorten the time needed to produce influenza vaccines, which enables strain selection closer to the start of flu season. GoF approaches to improve the yields of CVVs are uniquely capable of achieving this benefit in the immediate to near term relative to alternative approaches for shortening vaccine production timelines, for the reasons described above. Finally, a completely different mechanism for increasing the likelihood of vaccine match is to improve strain selection capabilities, which can be achieved through both GoF and alt-GoF approaches (see Section 9.8.5.3.1). Though promising, benefits in this area rely on scientific advancements and the expansion of influenza surveillance networks, thus both the extent and timescales of the benefits are uncertain. Notably, because this approach addresses different underlying gaps in existing vaccine development and production processes, research in this area has potential to complement the benefits that can be achieved by GoF research that shortens vaccine production timelines by increasing CVV yields.

9.6 Influenza Viruses: Benefits of GoF Research That Enhances Mammalian Adaptation and Transmissibility

9.6.1 Summary

This section describes the benefits of GoF research that is reasonably anticipated to enhance the infectivity and transmissibility of influenza viruses in representative animal models. Such GoF studies were found to generate scientific knowledge and inform surveillance of circulating animal influenza viruses, which has downstream impacts on decision-making about USG investments in pandemic preparedness initiatives, such as pre-pandemic vaccine development. Alt-GoF approaches that may generate similar benefits were also identified and analyzed. At present, GoF studies resulting in enhanced infectivity and transmissibility in mammals have unique benefits to scientific knowledge, surveillance, and pandemic preparedness, though full realization of GoF benefits to public health requires significant scientific advancements. Section 9.6 provides an overview of these benefits, including basic background and Supporting Information; a fully referenced and more thorough discussion of these benefits can be found in Section 15.3.

9.6.1.1 Benefits of GoF that Enhances Mammalian Adaptation and Transmissibility to Scientific Knowledge

- GoF approaches are:
  - Uniquely capable of proactively determining whether any animal influenza virus can evolve the capacity for airborne transmission in mammals.
  - Uniquely capable of providing in-depth information about the evolution of mammalian adaptation/transmissibility in any animal influenza strain, and of determining the order of acquisition of changes that are necessary and sufficient to enhance infectivity/transmissibility in mammals. However, laboratory results may not translate to adaptation of animal influenza viruses to humans in nature.
  - Uniquely capable of discovering novel genetic and phenotypic traits underlying mammalian adaptation and transmissibility in any virus strain, and of establishing a causal link between a particular trait and enhanced mammalian adaptation/transmissibility. However, results from cell culture or animal studies may not translate to human disease.

- Alt-GoF approaches are:
  - Limited to determining whether existing animal influenza viruses can efficiently transmit between mammals.
  - Uniquely capable of providing direct insight into the evolutionary mechanisms underlying adaptation of animal influenza viruses to humans, but cannot provide direct insight into the evolution of human transmissibility, as animal influenza viruses that efficiently transmit between humans do not exist in nature.
  - Uniquely capable of identifying novel genetic traits associated with adaptation of animal influenza viruses to humans, but cannot identify traits associated with human transmissibility, as animal influenza viruses that efficiently transmit between humans do not exist in nature.
  - Capable of identifying novel genetic traits in known phenotypes underlying mammalian adaptation and transmissibility using *in vitro*, virus free systems, but results may not be recapitulated in the context of the full virus.

9.6.1.2 Benefits of GoF that Enhances Mammalian Adaptation and Transmissibility to Surveillance

- GoF approaches:
  - Provide a foundation for the development of rapid assays for phenotypes underlying mammalian adaptation and transmissibility, which have potential to increase the quantity and timeliness of phenotypic information about animal flu viruses detected through surveillance. However, the success of this approach is subject to significant advancements in the state of knowledge about mechanisms underlying mammalian adaptation and transmissibility.
  - Are uniquely capable of strengthening the predictive value of molecular markers for mammalian adaptation and transmissibility, which can be used to infer phenotype from sequence. Use of molecular markers in lieu of or to corroborate phenotypic testing results could improve the quality, timeliness, and quantity of phenotypic information about animal flu viruses detected through surveillance. However, the success of this GoF approach is subject to significant advancements in the state of knowledge about the mechanistic basis of mammalian adaptation and transmissibility, and all sequence-based predictions must be experimentally validated.
  - Are critical for improving computational models for predicting phenotypes underlying mammalian adaptation and transmissibility based on sequence, which could improve the quantity and timeliness of phenotypic information about animal flu viruses detected through surveillance. However, the success of this approach is subject to significant advancements in the state of knowledge about the mechanistic basis of mammalian adaptation and transmissibility, and predictions must be experimentally validated.
- Alt-GoF approaches:
  - Have significant limitations for advancing the development of rapid assays for phenotypes underlying mammalian adaptation and transmissibility and for strengthening the predictive value of molecular markers for mammalian adaptation and transmissibility.
  - Are also critical for improving computational models for predicting phenotypes underlying mammalian adaptation and transmissibility based on sequence, but through the generation of different types of data that complement data generated through GoF approaches.

- Phenotypic assays for mammalian adaptation and transmissibility are uniquely capable of providing direct information about each complex phenotype under controlled conditions, but results may be delayed relative to the publication of viral sequences or, in the future, the generation of data about underlying phenotypes through rapid assays.

9.6.1.3 Benefits of GoF That Enhances Mammalian Adaptation and Transmissibility to Decision-Making in Public Health Policy

- GoF approaches:
  - Are uniquely capable of strengthening the predictive value of molecular markers for mammalian adaptation and transmissibility, which moderately influence pandemic risk assessments of circulating animal influenza viruses, relative to other types of data that are considered in the assessment. Pandemic risk assessments guide downstream decisions about investments in pre-pandemic vaccines, which will increase vaccine availability during a pandemic if a similar strain emerges to cause a pandemic.
  - Molecular marker data plays a relatively more important role when novel influenza viruses first emerge in human populations, when epidemiological data are scarce and virological data are not yet available. The ability to conduct a rapid risk assessment using molecular marker data can provide a three to four week head start on vaccine production.
  - Molecular marker data can guide selection of particular viruses to use as the basis of pre-pandemic vaccines, when multiple viruses have similar epidemiological and virologic characteristics.
- Alt-GoF approaches:
  - Epidemiological data are the most influential data in a pandemic risk assessment, but transmissibility can be difficult to assess in human populations, and epidemiological data may be scarce when novel viruses first emerge in human populations.
  - Virologic data strongly influences pandemic risk assessments, but the generation of virological data may be delayed relative to the publication of sequencing data when novel viruses emerge abroad due to shipping delays.
  - Other types of data, such as ecological data, also contribute to pandemic risk assessments but complement molecular marker data (GoF) by evaluating completely different aspects of pandemic potential.

9.6.2 Overview of GoF Research Landscape: Enhanced Infectivity and Transmissibility in Representative Animal Models

9.6.2.1 Serial Passaging of Viruses in Mammalian Cells or Animals

Serial passaging of viruses in mammalian cells in laboratory animals selects for viruses with enhanced growth in cells or enhanced infectivity to animals, respectively. This type of serial passaging experiment involves "forced" passaging, meaning that the experimenter directly transfers infected material, in the form of cell culture supernatant or homogenates of infected tissue, to the subsequent cell culture dish or animal. Forced serial passaging is carried out for two purposes: (1) to identify mutations that arise during adaptation of animal influenza viruses (i.e., avian and swine viruses) to mammals, which provides a

foundation for follow-up studies investigating the evolutionary mechanisms driving adaptation to mammalian hosts and the mechanistic basis of mammalian adaptation, and (2) to develop an mouse model for the study of a particular virus.

9.6.2.2 Serial Passaging of Viruses in Mammalian Cells or Animals with Selection for Transmission

Serial passaging of viruses in animals with selection for transmission leads to the generation of viruses with enhanced transmissibility in mammals. This type of serial passaging experiment can involve selection for contact transmission, during which the primary (directly inoculated) and secondary hosts are co-housed, or for airborne transmission, during which the primary and secondary hosts are separately housed in special isolator cages that prevent direct contact between animals but allow for air exchange between cages. These studies seek to identify mutations that are sufficient to enhance transmissibility, which provides a foundation for follow-up studies that investigate the mechanistic basis of transmissibility in mammals.

9.6.2.3 Forward Genetic Screen to Identify Genetic Traits That Enhance the Fitness/Transmissibility of Viruses in Mammals

Forward genetic screens involve random mutagenesis of genetic regions predicted to contribute to fitness/transmissibility or comprehensive reassortment of parental gene segments from two viruses, followed by characterization of the fitness or transmissibility of mutants in appropriate mammalian model systems to select for mutant viruses with enhanced fitness/transmissibility. Sequencing emergent viruses enables the identification of mutations or gene segments that enhance the fitness/transmissibility of viruses, which provides a foundation for follow-up studies that investigate the mechanistic basis of transmissibility in mammals.

9.6.2.4 Targeted Genetic Modification of Viruses to Introduce Traits That are Expected to Enhance Fitness/Transmissibility in Mammals

Targeted genetic modification of viruses, namely site-directed mutagenesis and/or reassortment, to introduce genetic traits that are expected to enhance the fitness/transmissibility of viruses followed by characterization of the fitness or transmissibility of mutants in appropriate mammalian model systems may lead to the generation of viruses with enhanced fitness/transmissibility in mammals. This approach is performed for two purposes: (1) to determine whether a previously characterized underlying genetic or phenotypic trait, such as a preference for binding to α2,6 sialic acid receptors, contributes to the complex phenotypes of mammalian adaptation or transmissibility and (2) to confirm that a particular mutation or gene segment is necessary and sufficient to enhance the fitness/transmissibility of viruses in appropriate model systems. Notably, genetic traits that are associated with mammalian adaptation/transmissibility may be discovered through GoF approaches or alt-GoF approaches. As above, this information provides a foundation for follow-up studies investigating the mechanistic basis of mammalian adaptation and transmissibility.

9.6.3 Identification of the Potential Benefits and Limitations of GoF Approaches

In this section, the potential benefits of GoF research that enhances mammalian adaptation and transmissibility in each benefit category listed in the NSABB Framework are discussed.

9.6.3.1 Benefits and Limitations of GoF Approaches to Scientific Knowledge

GoF approaches have potential to benefit several aspects of scientific knowledge about the ability of animal influenza viruses to adapt to efficiently infect and transmit between humans. In this section, the

ability of GoF approaches to address three key outstanding questions related to influenza virus adaptation and transmission in humans is evaluated:

- *Can* animal influenza viruses become transmissible between humans?
- *How* do animal influenza viruses adapt to and become transmissible between humans? What selective pressures drive adaptation and the evolution of efficient transmissibility, and what is the order of acquisition of new genetic/phenotypic traits that are needed for adaptation/transmissibility?
- *What* is the mechanistic basis of adaptation and transmission in humans? What viral factors are involved, and what phenotypic changes must occur in order for an animal influenza virus to adapt to efficiently infect, cause disease, and transmit in mammals?

Viral fitness and transmissibility are complex phenotypes that arise through the cumulative effects of multiple underlying phenotypes, such as specificity for a particular type of cell surface receptor and the ability to replicate within a particular temperature range. Because the property of transmissibility depends on phenotypes underlying both adaptation and transmission and because similar experimental approaches are used to study both complex phenotypes, GoF experiments that enhance adaptation and transmissibility are discussed together. Several phenotypes have been shown to be associated with mammalian adaptation and transmissibility. However, considerable gaps in knowledge remain about the molecular basis of each known phenotype and the role of each phenotype in adaptation/transmissibility. In addition, as-yet-undiscovered viral factors and phenotypic changes are likely to contribute to the acquisition of efficient transmissibility in mammals. Furthermore, the potential for animal influenza strains to evolve efficient transmissibility in humans is not understood.

9.6.3.1.1 Scientific Knowledge Gap 1: Can Animal Influenza Viruses Become Transmissible Between Humans?

Several GoF approaches can lead to the generation of transmissible viruses, including deliberate genetic modification of viruses and serial passaging of viruses in animals with selection for transmission. Collectively, these approaches definitively demonstrate that a virus can acquire the capacity to transmit between laboratory animals in an experimental setting. Notably, this approach can be applied to strains that have not yet caused infections in human populations as well as strains that have caused human infections but do not yet efficiently transmit in humans. The key limitations of this approach are that observations in animal models may not translate to humans and that the adaptive changes observed in the laboratory may not be possible in nature.

9.6.3.1.2 Scientific Knowledge Gap 2: How Do Animal Influenza Viruses Adapt to and Become Transmissible in Humans?

Serial passaging of animal influenza viruses in appropriate animal models to select for mammalian adaptation and transmission, a GoF approach, provides insight into the mechanisms underlying adaptation to mammals and the evolution of transmissibility. Sequencing of isolates at multiple stages of passaging enables determination of the order and rate of acquisition of adaptive traits, and follow-up studies elucidate how those genetic and phenotypic changes influence other viral phenotypes. Comparing the sequences and phenotypes of viral isolates from different tissues, at different time points during the course of infection, and between the primary (directly inoculated) and the secondary hosts can provide additional insight into the tissue-dependence of adaptation, the rate of intra- and inter-host adaptation, and the selection pressures and viral population dynamics during transmission, respectively. Notably, the

adaptive changes that occur in the lab environment under forced selection may not be relevant or possible during natural evolution, may not mimic adaptation and transmission in humans, and may selectively represent the evolutionary course possible for the limited number of viruses studied.

Serial passaging provides information about the genetic traits that are associated with the acquisition of enhanced fitness and transmissibility in mammals. However, to confirm which of these changes are *necessary* and *sufficient* to enhance fitness and transmissibility, targeted mutagenesis must be used to re-introduce mutations into parental strains followed by characterization of the infectivity/transmissibility of mutant strains. Targeted mutagenesis also enables determination of how the order of acquisition of genetic changes influences other viral phenotypes, such as replicative fitness, which has implications for the likelihood that these traits can arise in nature.

9.6.3.1.3 Scientific Knowledge Gap 3: What are the Genetic and Phenotypic Traits That Result in Adaptation and Transmission in Humans?

Several GoF approaches can be used to discover the genetic and phenotypic markers underlying mammalian adaptation and transmission of animal influenza viruses include:

- Targeted genetic modification to introduce novel genetic changes that are expected to contribute to adaptation and transmission in mammals by either site-directed mutagenesis or targeted reassortment (often between animal and human seasonal strains),
- Forward genetic screens involving random mutagenesis or comprehensive reassortment followed by selection for mammalian infectivity, transmissibility, or underlying phenotypes, and
- Serial passaging in appropriate animal models or mammalian cells to select for mammalian adaptive or transmissible traits.

Collectively, these approaches enable the identification of genetic changes that are sufficient to confer enhanced fitness in cell culture model systems or infectivity and transmissibility in animal models, which provides a foundation for follow-up biochemical, cell biological, and structural studies that elucidate associated phenotypic changes. Serial passaging has the potential to uncover *novel* genetic and phenotypic markers that contribute to adaptation/ transmissibility. In contrast, because forward genetic screens involving random mutagenesis typically focus on regions that are suspected or known to play a role in phenotypes underlying adaptation/transmissibility, this approach can discover novel *genetic* markers for adaptation/transmissibility only. The targeted genetic modification approach is limited to the investigation of genetic traits and underlying phenotypes that are suspected to contribute to adaptation/transmissibility (e.g., determining whether altering sialic acid receptor binding specificity contributes to transmissibility). Targeted genetic modification is also used to confirm that particular mutations or gene segments are *necessary* and *sufficient* to enhance infectivity or transmissibility in mammals. The use of *in vitro* model systems is limited to the investigation of phenotypes underlying adaptation and transmissibility, such as replicative fitness and sialic acid receptor specificity. Moreover, the results derived from these studies may not be recapitulated in the complex environmental pressures encountered in a host. The relevance of both *in vitro* and *in vivo* approaches depends on whether mechanisms underlying adaptation to cell culture and animal models are representative of those in humans, and results gleaned from the study of one or a few strains may not be recapitulated in different genetic contexts.

9.6.3.2 Benefits and Limitations of GoF Approaches to Surveillance

This section collectively evaluates the benefits of GoF research that enhances mammalian adaptation, transmissibility, or virulence to surveillance, as the strategies for monitoring the evolution of all three properties in circulating animal influenza viruses and the potential benefits of GoF approaches are similar.

GoF approaches that lead to the identification of genetic and phenotypic traits underlying mammalian adaptation, transmissibility between mammals, and virulence have the potential to inform the interpretation of wildlife, agricultural animal, and public health surveillance data. Specifically, GoF data has potential to improve three practices for evaluating the infectivity, transmissibility, and virulence of surveillance isolates: (1) inspecting sequences for the presence of molecular markers for mammalian adaptation, transmissibility, and virulence, (2) developing rapid assays for phenotypes underlying mammalian adaptation, transmissibility, and virulence, and (3) developing computational models for predicting underlying phenotypes. Information about the infectivity, transmissibility, and virulence of circulating animal influenza viruses is one aspect of evaluating their risk to human populations, which informs downstream decision-making related to public health preparedness for novel influenza outbreaks. The contribution of GoF data to pandemic risk assessments is discussed in detail in Section 9.6.3.3.2, below.

9.6.3.2.1 Introduction to Influenza Virus Surveillance: Current Practices and Limitations

Influenza surveillance is conducted in human and animal populations, including agricultural animals, companion animals, and wildlife. Collectively, the goal of this surveillance is to monitor the evolution of circulating animal influenza viruses, in order to detect the emergence viruses that pose a risk of emerging in human populations to cause a pandemic. Resources can then be dedicated to mitigating the risk factors associated with virus emergence, for example through community-level interventions at the animal-human interface, and to preparing for a potential emergence event, for example through the development of pre-pandemic vaccines.[584] Analysis of the phenotypic properties of individual surveillance isolates is a key aspect of assessing their pandemic potential. This section focuses on GoF benefits to that surveillance effort.

The WHO Global Influenza Surveillance and Response System (GISRS) serves as a central repository for data about animal influenza infections in humans. GISRS is a two-tiered surveillance and public health laboratory system.[585,586] A global network of National Influenza Centres (NICs) collect clinical specimens in their countries, perform preliminary analyses such as viral isolation and sub-typing, and forward specimens with suspect animal influenza infections to one of six WHO Collaborating Centres (WHOCCs) for further characterization.[587]

Multiple virus properties contribute to the likelihood that the virus will adapt to efficiently transmit in human populations and the potential consequences of that event, including whether the virus is adapted (or poised to adapt) to efficiently infect and transmit between humans and viral virulence. These properties can be directly measured in the laboratory or can be inferred from the genetic sequence based on the presence of molecular markers that have been linked to those phenotypes through previous research. In practice, due to caveats associated with both strategies, both are utilized. Two other approaches are in development but are not yet used in public health practice. The first involves the use of

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584 Ibid.
585 (2015p) Interview with Centers for Disease Control and Prevention representative.
586 WHO. Global Influenza Surveillance and Response System (GISRS). http://www.who.int/influenza/gisrs_laboratory/en/. Last Update Accessed December 7, 2015.
587 WHOCCs include the U.S. Centers for Disease Control in Atlanta, GA and St. Jude Children's Research Hospital in Memphis, TN.

rapid assays to measure phenotypes underlying mammalian adaptation, transmissibility, and virulence (i.e., versus evaluating the complex phenotype through animal experiments). The second involves computational modeling to predict phenotype from genotype, which incorporates experimental data about mutations that give rise to phenotypic changes, structural data, and other types of data. This section evaluates how GoF approaches can benefit surveillance by improving strategies for evaluating mammalian adaptation, transmissibility, and virulence through the use of molecular markers, the use of rapid phenotypic assays, and the use of computational models. First, the utility and limitations of traditional methods for laboratory evaluation of the infectivity, transmissibility, and virulence of viruses are evaluated. This information motivates the need for development of additional approaches that can provide information about these virus properties.

The pathogenicity and transmissibility of animal influenza viruses in mammals is typically evaluated in ferrets.[588] The strength of these assays is that they directly measure the complex properties of mammalian adaptation, transmissibility, and virulence. However, multiple shortcomings are associated with reliance on these assays. First, these assays are unable to assess when viruses have acquired underlying properties that are necessary but not sufficient to enhance infectivity, transmissibility, or virulence. Such knowledge about partial adaptation is of interest for pandemic risk assessments. Second, these assays require the use of surveillance isolates, which limits the number of viruses that can be subjected to phenotypic characterization.[589] Third, transmission and virulence testing in animals requires technical expertise and must be conducted under BSL-3 conditions, limiting the conduct of these assays to the six WHOCCs.[590] This restriction is problematic when logistical, political, and regulatory factors delay the shipment of virus samples from NICs and other field diagnostic laboratories to WHOCCs, thereby delaying the generation of phenotypic data.[591,592]

For the reasons listed above, the CDC has incorporated the use of molecular markers for phenotypes of concern into the pandemic risk assessment process to complement virologic data. Because the phenotypes of mammalian adaptation, transmissibility, and virulence are complex, arising from the interplay between multiple underlying phenotypes, this strategy involves inspecting sequences for markers that are casually linked to underlying phenotypes (e.g., altered sialic acid receptor binding specificity). Because a constellation of amino acid changes is needed for an animal virus to evolve to efficiently infect, transmit, and cause disease in people, molecular markers are considered collectively to determine the overall risk associated with a virus. Importantly, this process assumes that the complex phenotypes of mammalian adaptation, transmissibility, and virulence can accrue in a step-wise fashion, such that "partially adapted" viruses can persist in nature.

Influenza research experts agree that the state of this science does *not* enable accurate and reliable predictions of phenotype from genotype for complex phenotypes such as mammalian adaptation, transmissibility, and virulence. Multiple sources of scientific uncertainty limit current capabilities, which can be broadly grouped into two categories: (1) uncertainties related to the phenotypes underlying adaptation, transmissibility, and virulence and (2) uncertainties related to the genetic traits that alter underlying phenotypes.

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588 (2015c) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.
589 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136.
590 (2015c) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.
591 Ibid.
592 WHO (2011) Pandemic influenza preparedness framework for the sharing of influenza viruses and access to vaccines and other benefits.

Uncertainties related to phenotypes underlying mammalian adaptation, transmissibility, and virulence:

1. Weak linkage between underlying phenotypes and adaptation/transmissibility/virulence – that is, uncertainty in whether particular underlying phenotypes, such as altered sialic acid receptor binding specificity, are necessary for complex phenotypes, such as mammalian adaptation across many different virus strains.

2. Lack of knowledge about how underlying phenotypes interact to alter adaptation, transmissibility, and virulence (i.e., how to integrate the presence of multiple markers to appropriately determine overall risk).

3. Lack of knowledge about whether complex phenotypes can slowly accrue (i.e., whether partially adapted viruses can persist in nature) or whether the acquisition of efficient infectivity, transmissibility, and enhanced virulence in mammals is an "all-or-none" phenomenon.

Uncertainties related to the genetic traits that alter underlying phenotypes

1. Inability to predict whether a particular amino acid substitution will have similar phenotypic consequences in new genetic contexts.

2. Lack of knowledge about whether different amino acid substitutions at a particular amino acid position will have similar phenotypic consequences as known mutations.

3. Lack of knowledge about the mutational landscape that permits evolution of a complex phenotype – e.g., how many different sets of mutations enable the acquisition of airborne transmissibility? This knowledge gap influences whether the absence of a known marker is meaningful; if the mutational landscape is poorly understood, the "negative" strain could contain as-yet-undiscovered markers.

Collectively, these sources of uncertainty significantly compromise the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence.

Given the shortcomings associated with phenotypic assays and molecular marker data, the use of computational methods for sequence-based predictions of phenotypes underlying mammalian adaptation, transmissibility, and virulence has also been proposed. Although a variety of computational methods have shown promise for predicting phenotype from genotype, for those "known" phenotypes associated with adaptation/transmissibility, the accuracy of their predictions remains largely unknown.[593,594]

GoF approaches have potential to address shortcomings associated with the use of virological data, molecular markers, and computational methods to evaluate the infectivity, transmissibility, and virulence of animal influenza viruses in mammals, representing three different strategies for improving upon the status quo. The value of each strategy and the utility and limitations of GoF approaches for improving each strategy are discussed below.

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[593] (2015c) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.
[594] Russell CA *et al* (2014) Improving pandemic influenza risk assessment. *Elife* 3: e03883

9.6.3.2.2 Analysis of GoF Approaches That Support the Development of Rapid Phenotypic Assays

Strengths and weaknesses of using rapid phenotypic assays to inform pandemic risk assessments

Rapid assays to measure phenotypes underlying mammalian adaptation, transmissibility, and virulence could be performed in lieu of traditional evaluation of these complex phenotypes using ferrets. The development of rapid phenotypic assays holds promise for improving analysis of surveillance data for several reasons. First, the use of assays that are higher throughput than ferret testing will enable the phenotypic characterization of a larger number of viruses. Second, rapid phenotypic assays that require less technical expertise than ferret experiments are better suited for NICs, which would shorten the time lag between the initial detection and phenotypic characterization of a given virus. Thus, taken together, the development of rapid phenotypic assays has the potential to expand the quantity and the timeliness of phenotypic characterization data available for pandemic risk assessments. However, these assays will need to be carried out under BSL-3 conditions, which will limit the number of diagnostic laboratories that will be able to conduct the assays. (The majority of NICs do not have BSL-3 capabilities, though the number of NICs with BSL-3 capabilities or with access to BSL-3 labs has increased since 2005.)

In order for rapid phenotypic assays to be useful as proxies for mammalian adaptation, transmissibility, and virulence, the measured phenotype must be strongly linked to adaptation/transmissibility/virulence across many strain contexts. Additionally, interpretation of the results requires knowledge about how individual phenotypes contribute to overall pandemic risk, which relies on an understanding of how underlying phenotypes synergize to shape complex phenotypes. Gaps in scientific knowledge related to these two questions constrain the development and use of rapid phenotypic assays, as described above. As discussed in detail in Section 9.6.3.1.3, GoF approaches can provide insight into these scientific questions. The relevant findings are summarized below.

Summary – benefits of GoF approaches

GoF approaches represent the most efficient and effective approach for identifying novel *phenotypic* traits underlying mammalian adaptation, transmissibility, and virulence. Furthermore, targeted genetic modification of viruses to introduce genetic traits that alter underlying phenotypes is uniquely capable of demonstrating that a particular phenotype is causally linked to enhanced infectivity/transmissibility/virulence in mammals across multiple virus contexts. Additionally, the ability to alter phenotypes individually and in combination (i.e., through incorporation of varying sets of mutations) provides insight into how multiple underlying phenotypes interact to enhance infectivity, transmissibility, or virulence in mammals. This approach can also determine how an "intermediate" level of adaptation/transmissibility/virulence, i.e., acquisition of some but not all phenotypic traits that are required for viruses to efficiently infect, cause disease, and transmit in mammals, affects viral fitness, which may provide insight into whether such partially adapted strains can persist in nature. However, the major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to human disease in nature.

9.6.3.2.3 Analysis of GoF Approaches That Support the Use of Molecular Markers to Evaluate the Risk Posed by Circulating Animal Influenza Viruses

Strengths and weaknesses of using molecular marker data to inform pandemic risk assessments

The use of molecular marker data to evaluate the pandemic potential of animal influenza viruses has potential to improve the quantity and timeliness of phenotypic information about circulating animal influenza viruses. An increasing number of NICs and other diagnostic laboratories in developing countries have sequencing capabilities, and stakeholders involved in animal influenza surveillance stated

that viral genetic sequence data is currently the fastest and more reliable data generated by diagnostic labs in areas where viruses of concern are circulating.[595] Given the time needed for sample shipment to WHOCCs and ferret testing, the ability to assess the phenotypic properties of viruses based on sequence data can provide information before traditional phenotypic assays. Currently, most genetic surveillance data is generated by sequencing of viruses at WHOCCs.[596] Therefore, full realization of the benefits that can be derived from the use of molecular marker data will require an expansion of sequencing capabilities at diagnostic laboratories that comprise the "base" of the influenza surveillance system.

As described above, the current utility of molecular markers to the interpretation of genetic surveillance data is constrained by multiple sources of scientific uncertainty. Additionally, as knowledge about the phenotypes underlying mammalian adaptation, transmissibility, and virulence is incomplete, the discovery of additional molecular markers associated with novel underlying phenotypes would broaden the utility of this approach. As discussed in detail in Section 9.6.3.1.3, GoF approaches can provide insight into these scientific questions. The relevant findings are summarized below.

Summary – benefits of GoF approaches

GoF approaches represent the most efficient and effective approach for identifying novel genetic and phenotypic traits underlying mammalian adaptation, transmissibility, and virulence. Furthermore, targeted genetic modification of viruses to introduce genetic traits associated with mammalian adaptation/transmissibility/virulence is uniquely capable of establishing a causal link between a particular genetic or phenotypic trait and mammalian adaptation, transmissibility, or enhanced virulence across multiple strain contexts. In addition, GoF approaches, namely forward genetic screens, are uniquely capable of systematically exploring alternative mutational pathways for altering an underlying phenotype (e.g., changing sialic acid receptor binding specificity) in the context of whole virus. Finally, GoF approaches are also uniquely capable of providing definitive information about how multiple phenotypes synergize to promote mammalian adaptation, efficient transmissibility, and virulence. The major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to human disease in nature.

9.6.3.2.4 Analysis of GoF Approaches That Improve Predictive Models

Strengths and weaknesses of using computational models to inform pandemic risk assessments

As the use of computational models to predict phenotypes underlying mammalian adaptation, transmissibility, and virulence capitalizes on (and depends on) the availability of sequence data, the strengths and limitations of this approach are similar to those described above for the use of molecular marker data.

Existing computational models cannot reliably predict phenotypes underlying mammalian adaptation, transmissibility, and virulence based on sequence information. Additional experimental data is needed to appropriately parameterize models, and experiments must be conducted to validate the phenotypic predictions of models. GoF approaches can generate data that improves the accuracy of existing models.

Summary - benefits of GoF approaches

A variety of experimental data are needed to improve the accuracy of existing models, including data about mutations that do and do not give rise to phenotypic changes of interest. This data is critical for

[595] (2015e) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.
[596] Ibid.

building models that can account for the context dependence of genetic changes in influenza biology. GoF approaches (targeted mutagenesis and forward genetic screens) are uniquely capable of generating these data in the context of the full virus, although *in vitro*, virus free approaches can also be used. Finally, model predictions must be validated experimentally, and results feedback to improve model accuracy. GoF approaches are uniquely capable of validation model predictions in the context of the full virus.

9.6.3.3 Benefits and Limitations of GoF Approaches to Inform Policy Decisions

GoF approaches that lead to the identification of molecular markers for mammalian adaptation and transmissibility between mammals contribute to assessments of the pandemic risk posed by circulating animal influenza viruses, which are based on genetic surveillance data and several other types of data (e.g., epidemiologic data, phenotypic data, etc.). These assessments inform policy decisions related to public health preparedness for novel influenza outbreaks, including whether to develop pre-pandemic vaccines. Additionally, the demonstration that avian influenza viruses can evolve the capacity for more efficient transmission in mammals may, in and of itself, stimulate interest and investment in pandemic preparedness initiatives. This section evaluates the potential benefits of GoF approaches in both areas.

9.6.3.3.1 Benefits of "Proof of Principle" GoF Research That Demonstrates the Capacity of a Virus to Evolve More Efficient Transmissibility in Representative Animal Models

Researchers have suggested that the "proof of principle" demonstration that an animal influenza virus can evolve the capacity for airborne transmission in a laboratory setting, as a blunt indicator of the pandemic potential of the virus, could inform government interest and investment in pandemic preparedness initiatives. However, pandemic preparedness activities at the US CDC and ASPR, including BARDA, did not change in the wake of the laboratory demonstrations that H5N1 and H9N2 could evolve the ability to transmit via the airborne route between ferrets in 2012 and 2009, respectively, suggesting that this is not a real benefit.[597,598,599] USG representatives involved in pandemic preparedness indicated that the response to the demonstration that an animal virus that has not yet caused human infections can evolve the capacity for airborne transmission would also be minimal, due to the lack of certainty about whether laboratory results translate to humans in nature.[600] If the virus were known or suspected to be circulating in animal populations in the US, enhanced surveillance might be undertaken to better understand the prevalence and geographic distribution of the virus in nature. However, the result would be highly unlikely to trigger investments in pre-pandemic vaccine development.

9.6.3.3.2 Benefits of GoF Research That Informs Pandemic Risk Assessments

The second mechanism through which GoF approaches can benefit pandemic preparedness planning is through pandemic risk assessments, downstream of GoF benefits to surveillance. As discussed in Section 9.6.3.2, GoF approaches have potential to benefit virological surveillance (i.e., by supporting the development of rapid phenotypic assays) as well as genetic surveillance (i.e., by strengthening the predictive value of molecular markers for phenotypic properties of concern and by improving computational models for predicting phenotype from genotype). The use of molecular markers for phenotypic properties of concern is currently incorporated into the risk assessment process, as described in detail below. As neither rapid assays nor robust computational models for relevant phenotypes exist, how results from notional future assays/models would be considered in risk assessments is uncertain.

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597 Imai M *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486: 420-428

598 Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

599 (2015k) Interviews with CDC, ASPR, and BARDA representatives.

600 (2015j) Interviews with CDC and BARDA representatives.

Thus, the potential benefits of rapid phenotypic assays or computational models to pandemic risk assessments is not formally evaluated in this section, but a discussion of how results from either could contribute to the risk assessment process is provided at the end of the section.

This section analyzes the value of using molecular marker data relative to other types of data that are considered in the pandemic risk assessment process, which provides an "upper bound" to the public health benefits that can be achieved through GoF improvements to surveillance. First, to provide context for this analysis, current strategies for pandemic risk assessments are reviewed, and shortcomings in existing processes are highlighted.

Background – pandemic risk assessment and strategies for decision-making about investments in pandemic preparedness

The US government undertakes influenza pandemic preparedness activities that aim to bolster US capabilities for rapid detection of novel influenza events and to limit the spread of disease, death, and potential societal impacts if/when the next influenza pandemic occurs.[601] In particular, the development of pre-pandemic vaccines is a key aspect of pandemic preparedness because influenza vaccination is the primary public health strategy during outbreaks.[602] As resources for pandemic preparedness efforts are limited, a major challenge is determining how resources for the development of pre-pandemic vaccines and other preparedness activities should be allocated. To that end, the CDC, in collaboration with subject matter experts in influenza virology, diagnosis, epidemiology, ecology, and laboratory research in animal and human influenza, developed a framework for assessing the relative risk posed by emerging influenza viruses and an accompanying tool – the Influenza Risk Assessment Tool (IRAT). Those results then inform prioritization of resources for preparedness efforts directed at particular strains/sub-types (e.g., vaccine development).

The IRAT provides a formal method for evaluating the relative risk posed by different emerging influenza strains (e.g., H5N1 versus H7N9).[603,604] This method is based on SME input about risk elements that govern the likelihood that a particular strain will adapt to efficiently transmit in human populations and the expected public health consequences of that emergence event. These risk elements can be broadly grouped into four categories:

- Elements relating to the properties of the virus (e.g., transmissibility and virulence),
- Elements relating to the attributes of host populations (e.g., the degree of pre-existing immunity),
- Elements relating to epidemiological, and
- Elements relating to ecological factors (e.g., the extent of human infections and the prevalence and geographic distribution of the virus in animal populations).

Selected elements will be described in more detail below. Risk elements pertaining to the properties of the virus are informed by virological data (e.g., transmission studies in ferrets) and by genomic data, including molecular marker data (e.g., whether molecular markers associated with enhanced transmissibility in ferrets are present in the viral genetic sequence). Individual risk elements are weighted, based on SME input about their relative contribution to the likelihood and expected consequences of

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601 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

602 Ampofo WK *et al* (2013b) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

603 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

604 Trock SC *et al* (2012) Development of an influenza virologic risk assessment tool. *Avian diseases* 56: 1058-1061

emergence of particular strains, and all elements are considered collectively to determine an overall risk score. Notably, although not all policy decisions related to pandemic preparedness rely on formal risk assessments, the same factors are considered when informally evaluating the risks posed by emerging influenza viruses.

Potential Benefits of GoF to pandemic risk assessments: utility and limitations of using molecular marker data

GoF approaches have potential to improve the accuracy, timeliness, and quantity of phenotypic information generated by inspecting sequences for the presence of molecular markers for mammalian adaptation, transmissibility, and virulence, as described in detail in Section 9.6.3.2.3. This section focuses on the utility and limitations of molecular marker data to the pandemic risk assessment process.

Molecular marker data

Molecular markers for phenotypes underlying mammalian adaptation, transmissibility, and virulence are considered as part of the "genomic variation" risk element (which also incorporates consideration of reassortment). As described above, these analyses complement results from laboratory-based phenotypic assays. The major strength of this analysis is that sequence data are now the fastest, most reliable data produced at NICs and other field laboratories where animal influenza viruses of concern are circulating, enabling evaluation of viruses prior to the generation of virological data in the laboratory.[605] However, the predictive value of molecular markers is compromised by significant sources of scientific uncertainty, as described above. Because of these uncertainties, molecular marker data contributes moderately to the risk assessment, relative to other factors. For example, in the three-virus relative risk assessment referenced above, findings related to epidemiology risk elements were about six-fold more important than findings in the genomic variation risk element.

9.6.3.3.3 Public Health Impacts of Pandemic Risk Assessments

Pandemic risk assessments are carried out to help prioritize resources for investments in pre-pandemic vaccine development. Risk assessments may also guide investments in other pandemic preparedness initiatives, such as testing the efficacy of antivirals against high-risk viruses. GoF approaches aid decision-making downstream of pandemic risk assessments insofar as GoF-derived data contributes to the pandemic risk assessment process. Additionally, GoF approaches can be used to select particular viruses to be used as the basis of pre-pandemic vaccine strains.

Pre-pandemic vaccine development

Because existing influenza vaccines are strain-specific, pre-pandemic vaccines are developed to target particular groups of high-risk strains. Depending on the overall level of risk associated with a particular virus, the US government will fund development of a pre-pandemic vaccine through various stages of the vaccine production pipeline. Each of the following steps requires an escalating expenditure of resources: CVV development, conduct of pre-clinical vaccine studies in animals, manufacture of clinical trial lots of vaccine, conduct of human clinical trials, stockpiling of vaccine, and priming the population against the novel influenza virus. Collectively, these investments will increase the availability of vaccines during a pandemic by shortening vaccine production timelines.[606] Although the pre-pandemic vaccine strain is unlikely to exactly match the strain that emerges to cause a pandemic, use of adjuvants and prime-boost regimens broaden the protection that can be achieved using a strain-specific vaccine, such that pre-

[605] (2015h) Interview with CDC Representative.
[606] (2015c) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

pandemic vaccines are highly likely to provide some level of protection against infection with a similar strain.[607,608] Notably, resources limit the scope of the USG's investment in pre-pandemic vaccines, highlighting the need for strategies to prioritize vaccine development for the many influenza viruses circulating in nature that have spilled over into human populations.

GoF data may play a role in the decision to develop a CVV for an animal influenza virus by contributing to the pandemic risk assessment process, particularly when new viruses first emerge in human populations and sequence data are available before other types of virologic data. Once the decision is made to develop a CVV, multiple strains may be available to serve as the basis for the CVV. In the event that these strains have similar epidemiological and virological characteristics, the presence and type of molecular markers for mammalian adaptation, transmissibility, and virulence can serve to differentiate between strains. This application of GoF data enables more granular decision-making than would have been possible based on other data sources alone, which is valuable because resource limitations constrain that number of CVVs that can be produced.[609]

Both animal influenza viruses isolated from human infections as well as animal influenza viruses that have not yet caused human infections can be subjected to a risk assessment (formally or informally). However, because of the expense involved in each step of pre-pandemic vaccine production, none of the above steps are likely to be undertaken unless multiple human infections have occurred.[610] As a result, although GoF approaches may aid the interpretation of surveillance data from animals, this proximal benefit will not lead to downstream investments in pre-pandemic vaccine development but rather is limited to deepening understanding of the risk associated with particular viruses.

A completely different strategy for increasing the availability of vaccines during a pandemic is by shortening vaccine production timelines. GoF research that enhances virus production enables the development of higher-yield CVVs, which shortens vaccine production timelines by increasing the rate of bulk antigen production. Although this research can be immediately applied to improve vaccine production, this strategy provides the greatest benefit to the production of vaccines using poor-growing CVVs. However, as any strain may unexpectedly generate a low-yield CVV, such as the 2009 H1N1 pandemic strain, this benefit could significantly alleviate morbidity and mortality in the event that future pandemic strains are also grow poorly.

Field investigations of clusters of zoonotic influenza infections abroad

The CDC participates in missions to investigate zoonotic influenza cases or clusters of concern abroad, in conjunction with the WHO, OIE, Food and Agricultural Organization of the United Nations (FAO), and local Ministries of Health. The goal of these missions is to supplement foundational surveillance with in-depth investigations of ecological and environmental factors that may be contributing to spillover, such as sources of human exposure and the extent to which the viruses are circulating in local animal populations.[611] Collectively, these data improve understanding of the risk posed by the zoonotic influenza virus in that environment, which informs decision-making about other prevention and preparedness activities (such as whether to develop a pre-pandemic CVV). Recent examples include missions to Cambodia to investigate an abrupt rise in human H5N1 infections in 2013, to China in 2013 to investigate

607 Ibid.
608 (2015d) Influenza Vaccines. Interviews with Public Health Professionals Involved in Preventing and Responding to Influenza Outbreaks.
609 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.
610 (2015b) Interview with USG representative involved in pandemic risk assessment and decision-making about investments pandemic preparedness initiatives.
611 Davis CT *et al* (2014) Use of highly pathogenic avian influenza A(H5N1) gain-of-function studies for molecular-based surveillance and pandemic preparedness. *MBio* 5

the initial wave of H7N9 human infections, and to Cairo, Egypt in March of 2015 to investigate the dramatic increase in the number of human cases of H5N1 infection recorded at the end of 2014 leading into the first few months of 2015.[612,613] The decision to send a CDC team abroad is informed by an assessment of whether the sequences of human isolates contain molecular markers for mammalian adaptation, virulence, and transmissibility. Similar to a formal risk assessment, this decision is driven by epidemiologic data, but the presence of molecular markers of concern adds value by increases certainty in decision-making. In addition, consideration of molecular marker data may stimulate increased attention to investigations of the local animal population and human interactions with infected animals, undertaken to better understand how ecological and environmental factors are influencing the evolution of the virus in that area.

9.6.3.4 Vaccines

GoF approaches have the potential to benefit the development of pre-pandemic vaccines. Specifically, pandemic risk assessments, which can be informed by GoF research (see Section 9.6.3.3), may trigger the development of candidate vaccine viruses based on high-risk viruses, as well as subsequent stages of the pre-pandemic vaccine production pipeline (e.g., manufacturing of clinical lot material, conducting human clinical trials, and stockpiling vaccine).

9.6.3.5 Therapeutics

A lack of knowledge about whether existing therapeutics will be effective against future pandemic strains hampers preparedness planning. GoF-generated viruses that are transmissible between ferrets may mimic pandemic variants of that HA subtype better than wild type viruses. Thus, testing whether existing therapeutics are capable of mitigating disease caused by GoF strains could inform pandemic preparedness planning. Researchers have also suggested that these experiments could stimulate the development of new therapeutics, in the event that existing therapeutics are found to be ineffective against GoF strains. However, the relevance and utility of this information is severely constrained by several sources of uncertainty, including a lack of knowledge about whether ferret-transmissible viruses are more transmissible in humans, whether laboratory-generated transmissible viruses behave similarly to those that could arise in nature, and other factors. Given this uncertainty, dedication of resources to developing therapeutics targeting hypothetical future pandemic viruses is unlikely. Thus, this putative benefit to the development of therapeutics is not considered in this report.

9.6.3.6 Diagnostics

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[614,615]

9.6.3.7 Economic Benefits

Pandemic risk assessments inform prioritization of resources for pandemic preparedness. Specifically, evaluating the relative risk posed by different influenza viruses helps decision-makers allocate limited funds to pandemic preparedness efforts, such as the development of pre-pandemic vaccines targeting

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612 Ibid.

613 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

614 New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

615 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

high-risk viruses. This prioritization may improve the efficiency of government spending on influenza pandemic preparedness. Economic benefits were not explicitly evaluated in this report.

9.6.4 Identification of the potential benefits and limitations of alt-GoF approaches that provide similar potential benefits to the GoF approaches being examined

In this section, an overview of alternative (alt-GoF) approaches that yield the same or similar benefits as the GoF approaches described above is provided. Two types of alt-GoF approaches are reviewed: (1) alternative experimental approaches that can provide the same or similar scientific information as GoF experimental approaches, and (2) alternative scientific and technical innovations that can yield the same public health benefits as GoF approaches, but through different mechanisms. For each approach, the scientific outcomes of the approach and how that information leads to similar benefits as GoF approaches are described.

9.6.4.1 Potential Benefits and Limitations of Alt-GoF to Scientific Knowledge

9.6.4.1.1 Scientific Knowledge Gap 1: Can Animal Influenza Viruses Become Transmissible Between Humans?

Characterizing the transmissibility of wild type isolates in representative animal models represents an alternative approach for addressing whether animal influenza viruses display the capacity for transmission between mammals. However, this approach is inherently reactive – that is, it can effectively answer whether a virus is transmissible but cannot shed light on whether a virus has the potential to become transmissible. Additionally, observations in animal models may not translate to humans.

9.6.4.1.2 Scientific Knowledge Gap 2: How Do Animal Influenza Viruses Adapt to and Become Transmissible in Humans?

Several alt-GoF approaches can address how influenza viruses evolve to efficiently infect and transmit in humans. First, the comparison of sequences from closely related human and animal isolates enables the identification of the origin and evolutionary rate of genetic changes among circulating viruses, which can provide information on selection pressures and diversity among viruses in different hosts. That this approach examines the natural course of adaptation and underlying mechanisms of infection and transmission of viruses *in humans* is a strength relative to GoF approaches and other alternatives that depend on the suitability of animal models in an artificial environment as representative of human disease. However, this approach suffers from several significant limitations. The use of comparative sequence analysis is feasible only if human-adapted and transmissible viruses have arisen in nature, but to date, animal influenza viruses have limited capacity to infect and transmit in humans. Analysis of the few animal-origin spillover infections may however inform evolution of adaptive traits. The success of this approach is significantly constrained by the quality and availability of genetic surveillance data. In particular, the noisiness of comparative sequence analysis due to high genetic diversity among influenza viruses practically limits this approach to the examination of genetic regions known to be important for adaptation and transmissibility, unless precursor-spillover paired strains can be identified (which is rare). Additionally, the fact that the surveillance record is static and incomplete limits the depth of evolutionary information that can be gleaned from this approach.

Analysis of viruses that have emerged from avian or mammalian reservoirs to become transmissible in other mammalian species represents another surveillance-based approach for studying the mechanisms underlying adaption to mammals during interspecies transmission. The recent emergence of animal transmissible influenza viruses in other mammals (e.g., an avian-origin H3N2 canine influenza virus that emerged in dogs in the mid-2000s) enables the study of the full evolutionary pathway for cross-species

acquisition of efficient transmissibility. This approach is subject to the same limitations as comparative sequence analysis of human and animal isolates, with the additional caveat that adaptation to other mammals may occur through different pathways and mechanisms than in humans.

Phenotypic characterization of wild type viruses by evaluating infectivity and transmissibility in appropriate model systems is another alt-GoF approach for studying the evolution and mechanisms of adaptation/transmissibility. This approach allows the generation of in-depth information about evolutionary mechanisms; however, relevant evolutionary changes may not occur during a single round of transmission. Moreover, any animal influenza viruses that are highly attenuated in representative animal models or are incapable of establishing infection are not suitable for this approach. Finally, this approach depends on the suitability of the animal models used for characterization.

9.6.4.1.3 Scientific Knowledge Gap 3: What Are the Genetic and Phenotypic Traits That Result in Adaption and Transmission in Humans?

Several alt-GoF approaches can be used to uncover genetic and phenotypic traits underlying adaptation and transmission in mammals. First, comparing the sequences of human and animal isolates enables the identification of genetic changes that are associated with human adaptation and transmissibility. This approach has the potential to directly identify human-adaptive traits and may be more likely to uncover conserved traits through analysis of a large number of strains. However, the fact that no animal influenza viruses that efficiently transmit in humans have been observed in nature precludes the use of this approach to identify mechanisms underlying transmissibility. For the discovery of mammalian adaptive traits, the success of this approach is constrained by the quality and availability of surveillance data. In addition, the extensive genetic diversity within circulating virus populations and among viruses isolated from humans makes discerning distinct genetic traits that are likely to contribute to fitness and transmissibility in humans relative to animals difficult. Namely, the "noise" associated with sequences comparisons obscures the discovery of relevant features that distinguish human versus animal isolates, which practically limits this approach to the investigation of traits or regions previously known to be important for adaptation.

Comparing the sequences of evolutionarily related isolates from different animal species represents another surveillance-based approach for identifying genetic traits that are associated with mammalian adaptation and transmissibility. Importantly, because avian-origin flu viruses that are airborne or contact transmissible exist in circulation in several mammals including seals, horses, and dogs, this approach is currently feasible for the study of transmissibility. In addition to the limitations above, mechanistic insight gleaned through this approach may not translate to the adaptation of animal influenza viruses to humans.

Phenotypic characterization of wild type viruses in appropriate animal models is another alt-GoF approach that complements the use of surveillance data to study mechanisms underlying mammalian adaptation and transmissibility. Specifically, comparing the sequences of wild type viruses with varied levels of fitness and transmissibility enables the identification of genetic traits associated with fitness/transmissibility. This approach is limited to the study of viruses that can productively infect and transmit between animal models for adaptation/transmission. Notably, very few natural animal-origin viruses are capable of transmission in ferrets and many are not able to efficiently cause disease in representative animal models. Genetic and phenotypic traits uncovered through this approach may not translate to human-adapted viruses and may only be applicable to the limited number of strains analyzed.

Loss of Function (LoF) approaches, genetic screens that utilize random mutagenesis or targeted genetic modification to identify genetic changes that attenuate fitness and transmission in mammals, can provide information about genetic and phenotypic traits that contribute to transmissibility. Targeted LoF can also

be used to confirm necessary genetic or phenotypic traits by determining that mutations attenuate fitness or transmission, but cannot identify traits that lead to enhanced transmission. This approach suffers from several significant limitations. First, LoF studies can be performed only using transmissible seasonal or pandemic viruses, and insights may not translate to animal influenza viruses. Second, because of the high mutation rate of influenza viruses, LoF mutations that attenuate transmissibility may revert during the single round of passage that is needed to characterize the transmissibility of the mutants (which represents a selection step). Third, because many mutations attenuate transmission for trivial reasons, for example mutations that compromise viability, discovering traits that directly contribute to transmissibility may be difficult using a LoF approach. Finally, although in principle LoF screens can be performed after random mutagenesis to discover new genetic elements important for transmission, the resource intensive nature of transmission studies in ferrets practically limits these studies to the investigation of a few, known targets.

Several *in vitro* virus-free methods can be used to investigate phenotypes underlying adaptation and transmissibility. Comparative sequence analysis of viral proteins with different phenotypic properties can then enable the identification of mutations that are associated with relevant phenotypic changes, while forward genetic screens can be used to identify novel *genetic* traits that contribute to underlying phenotypes. Additional characterization involves the use of biochemical assays (e.g., characterizing the acid stability of the HA protein) and crystallographic resolution of the structures of virus-host protein complexes can provide insight into the functional and biophysical basis of underlying phenotypes. The use of targeted modification of viral gene segments in isolation can also effectively confirm the *necessary* and *sufficient* genetic traits that alter an underlying phenotype. Though the simplicity and relatively high-throughput nature of these methods renders them appealing as a screening approach for the discovery and confirmation of *novel* genetic traits that contribute to adaptation/transmissibility, these approaches are inherently limited to the characterization of genetic traits and phenotypes previously identified in other experiments. An additional drawback is that results gleaned from studying the behavior of a viral protein or phenotype in isolation may not be recapitulated in the context of the full virus or *in vivo*. Moreover, although fairly rapid phenotypic assays have been developed for the study of phenotypic traits known to be associated with adaptation/transmissibility, assays to study phenotypic traits may be unreliable or unavailable for future phenotypes of interest.

Structure-based modeling approaches, an *in silico* method, may also be used to predict the effects of mutations on phenotypes underlying adaptation/transmissibility. This approach is critically limited by the capabilities and accuracy of existing models, and as such any conclusions may not be consistent in the context of the full virus.

Finally, several alt-GoF approaches focus on identifying host factors and host-virus interactions that are associated with mammalian adaptation, which may provide indirect insight into viral mechanisms underlying cross-species adaptation. Specifically, *in vitro* proteomic (e.g., mass spectrometry) and genomic screens (e.g., RNAi screen) utilizing both virus-free and cell culture-based infection systems are used to identify host factors that interact with virus proteins of interest or that are critical for underlying phenotypes, such as viral replication. These approaches complement the identification of viral proteins/phenotypes underlying adaptation to new hosts. However, the breadth of proteomic approaches is limited in that screens typically focus on a single viral protein, and both genomic and proteomic screens can identify host proteins that may not be functionally relevant or may play minor roles in the viral life cycle.

Another type of alternative approach involves the use of attenuated viruses for GoF methods as a risk mitigation strategy. Four types of attenuated viruses have been used for such studies: (1) reassortants with surface protein gene segments from seasonal influenza viruses, to which the general population has pre-existing immunity, (2) reassortants with lab-adapted viruses (e.g., PR8), (3) strains which have virulence factors altered or deleted (e.g., deletion of the multi-basic cleavage site in HPAI HA sequences), and (4)

strains which have incorporated binding sites for microRNAs (miRNAs) that are expressed in humans but not an animal model of interest, and therefore are replication-competent in experimental animals but not humans (termed "molecular biocontainment").[616]

Results gleaned through use of attenuated viruses may be of limited informational value because complex, multi-genic traits depend on genetic context (a phenomenon called epistasis), and results may not be recapitulated in the context of the wild type virus. Differences in disease pathogenesis, which critically influences the biological processes of adaptation and transmission, further compromise the relevance of results gained through the use of attenuated strains. Finally, although the microRNA-based molecular biocontainment strategy is considered promising by the influenza research community, only two such engineered strains have been created to date, neither of which has been extensively characterized with respect to infection and transmission dynamics in ferrets or permissive cell lines. Additional research is needed to determine whether and to what extent the engineered strains serve as functional proxies for their cognate WT strains, before these strains can be widely used to probe scientific questions about mammalian adaptation and transmission of influenza viruses. In addition, because the purpose of this miRNA strategy is to restrict virus replication in people, this strategy is not suitable for studies using human cell lines, limiting its utility for *in vitro* studies investigating phenotypes underlying mammalian adaptation and transmissibility.

9.6.4.2 Benefits and Limitations of Alt-GoF Approaches to Surveillance

Akin to Section 9.6.3.2, this section evaluates the benefits of alt-GoF approaches for evaluation of the infectivity, transmissibility, and virulence of animal influenza viruses detected through surveillance. These virus properties may be directly measured in the laboratory or can be inferred from the viral genetic sequence based on the presence of molecular markers that have been linked to those phenotypes through previous research. Two other approaches are in development but are not yet used in public health practice include the use of rapid assays to measure phenotypes underlying mammalian adaptation, transmissibility, and virulence and computational modeling to predict phenotype from genotype. Each of these methods has shortcomings which can be addressed by GoF approaches, as detailed in Section 9.6.3.2. The ability of alt-GoF approaches to similarly address these shortcomings is evaluated below.

9.6.4.2.1 Analysis of Alt-GoF Approaches That Support the Development of Rapid Phenotypic Assays

In order for rapid phenotypic assays to be useful as proxies for mammalian adaptation, transmissibility, and virulence, the measured phenotype must be strongly linked to adaptation/transmissibility/virulence across many strain contexts. Moreover, interpretation of the results requires knowledge about how individual phenotypes contribute to overall pandemic risk, which relies on an understanding of how underlying phenotypes synergize to shape complex phenotypes. Gaps in scientific knowledge related to these two questions constrain the development and use of rapid phenotypic assays. As discussed in detail in Section 9.6.4.1.3 and Section 9.7.4.1.1, alt-GoF approaches can provide limited insight into these scientific questions. The relevant findings are summarized below.

Characterization of wild type viruses provides limited insight into phenotypic traits underlying mammalian adaptation and transmissibility because animal influenza viruses that efficiently infect and transmit in humans do not exist in nature. However, characterizing the constellation of underlying phenotypes present in a large number of wild type viruses (e.g., sialic acid receptor binding specificity, HA stability, optimal temperature for polymerase activity, etc.) is uniquely capable of providing insight

[616] Langlois RA *et al* (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844-847

into whether viruses that have a subset of the properties that are necessary for enhanced infectivity, transmissibility, or virulence can persist in nature.

LoF approaches have limited utility for broad and unbiased identification of phenotypic traits that contribute to transmissibility and pathogenicity due to their inefficiency and the fact that mechanisms underlying transmissibility of seasonal/pandemic viruses may not translate to animal influenza viruses. Though LoF approaches can be used to causally demonstrate that a particular phenotype is necessary for efficient transmissibility and enhanced virulence, this approach cannot be used to understand how multiple phenotypes synergize to enhance infectivity, transmissibility, or virulence. This information critically informs how results from multiple phenotypic assays should be integrated to evaluate overall pandemic potential. Surveillance-based approaches, including comparison of human and animal isolates, comparison of sequences spanning avian to mammalian adaptation events, and comparison of viral isolates with varying levels of virulence are limited to the study of previously known traits and provide associative data. Notable exceptions include the analysis of precursor/spillover pairs, for the study of adaptation/transmissibility, and analysis of viral isolates over the course of infection in a single patient, for the study of virulence. However, the availability of both types of paired isolates is low. In addition, surveillance-based approaches cannot provide insight into phenotypes underlying transmissibility because animal influenza viruses that efficiently transmit in humans do not exist in nature. *In vitro*, virus free approaches, which involve the study of known phenotypes in isolation, cannot provide information about the functional relationships among underlying phenotypes or between underlying phenotypes and adaptation/transmissibility.

9.6.4.2.2 Analysis of Alt-GoF Approaches That Support the Use of Molecular Markers to Evaluate the Risk Posed by Circulating Animal Influenza Viruses

As described previously, the current utility of molecular markers for the interpretation of genetic surveillance data is constrained by multiple sources of scientific uncertainty. As discussed in detail in Section 9.6.4.1.1 and Section 9.6.4.1.3, alt-GoF approaches can provide some insight into relevant scientific questions that strengthen this approach. The relevant findings are summarized below.

In sum, alt-GoF approaches, namely characterization of wild type viruses, are uniquely capable of demonstrating whether partially adapted viruses exist in nature, which provides insight into whether complex phenotypes such as adaptation, transmissibility, and virulence can accrue in a step-wise fashion (an underlying assumption of the use of molecular markers to evaluate pandemic risk). However, alt-GoF approaches have significant limitations for addressing other relevant knowledge gaps at the phenotypic level, in particular strengthening the linkage between underlying phenotypes and mammalian adaptation/transmissibility/virulence.

Alt-GoF approaches can provide some insight into the scientific knowledge gaps about the *genetic* traits underlying mammalian adaptation, transmissibility, and virulence that compromise the application of molecular marker data to surveillance. Characterization of wild type viruses provides limited insight into genetic traits underlying mammalian adaptation and transmissibility because animal influenza viruses that efficiently infect and transmit in humans do not exist in nature. LoF approaches have limited utility for broad and unbiased identification of novel genetic traits that are necessary for transmissibility or enhanced virulence due to their inefficiency and the fact that mechanisms underlying transmissibility of seasonal/pandemic viruses may not translate to animal influenza viruses. Surveillance-based approaches, including comparison of human and animal isolates and of sequences spanning avian to mammalian adaptation events, have limited utility for the discovery of *novel* genetic traits associated with adaptation/transmissibility/virulence due to the high genetic diversity of influenza viruses and shortcomings in the quality and availability of surveillance data. However, surveillance-based approaches have several unique strengths for validating the functional consequences of particular markers.

Comparison of human and animal isolates or of human isolates with varying levels of virulence is uniquely capable of providing direct insight into traits associated with human adaptation and virulence across multiple strain contexts. These traits can be considered "causally" linked if a large enough number of sequences are compared. Notably, this approach cannot be used to validate markers associated with enhanced transmissibility because animal influenza strains that transmit efficiently between humans do not exist in nature. The high-throughput nature of *in vitro*, virus free approaches relative to animal experiments renders them appealing for the discovery of additional mutations that give rise to particular phenotypic changes (through forward genetic screens) and for validating the function of particular markers in new genetic contexts. However, results may not be recapitulated *in vivo*, in the context of the full virus.

9.6.4.2.3 Analysis of Alt-GoF Approaches That Improve Predictive Models

Another strategy for evaluating infectivity, transmissibility, or virulence involves the use of computational models that predict phenotypes underlying mammalian adaptation, transmissibility, and virulence based on viral sequences. However, existing computational models cannot reliably predict underlying phenotypes based on sequence information.

A variety of experimental data are needed to improve the accuracy of existing models, including data about mutations that do and do not give rise to phenotypic changes of interest. This data is critical for building models that can account for the context dependence of genetic changes in influenza biology. Alternative experimental approaches cannot provide this information. In addition, model predictions must be validated experimentally, which feeds back to improve model accuracy. Alternative approaches can only test model predictions using *in vitro*, virus-free systems. As results may not be recapitulated in the context of the full virus, this approach is of limited utility for improving the quality of models.

Experimental data about the biophysical basis of underlying phenotypes, such as crystallography data and measurements of HA binding affinities to α2,6 and α2,3 sialoglycans, is also needed to improve existing models. This information can only be generated using alt-GoF approaches.

9.6.4.3 Benefits and Limitations of GoF Approaches to Inform Policy Decisions

GoF approaches have potential to benefit decision-making in public health policy by contributing to pandemic risk assessments, which guide investments in pandemic preparedness initiatives, as described in Section 9.6.3.3.2. This section evaluates how alternative types of data contribute to pandemic risk assessments, thereby similarly benefitting downstream decision-making related to pandemic preparedness. Three alternative data sources are described: virologic data, epidemiologic data, and ecological data.

9.6.4.3.1 Potential Benefits and Limitations of Alt-GoF Approaches to Pandemic Risk Assessments

Potential Benefits and Limitations of Alternative Pandemic Risk Assessment Factors: Virologic data

The relative strengths and weaknesses of using virological approaches to characterize the phenotypic properties of surveillance viruses were discussed extensively in Section 9.6.3.2.1. This section evaluates the utility and limitations of virologic data in the context of the overall pandemic risk assessment.

Several risk elements rely on laboratory data: receptor binding (preference for "human-like" α2,6 sialylated receptors, "avian-like" α2,3 sialylated receptors, or dual specificity), transmission in animal models, antiviral resistance, disease severity in animal models, and antigenic relationship between virus

and existing CVVs/vaccines.[617,618] Although epidemiologic measurements also provide information about the severity and transmissibility of a virus, these phenotypes are difficult to measure accurately in nature, especially when a virus first emerges in human populations and epidemiological data are scarce. As performing human transmission and virulence studies using novel influenza viruses would be unethical, laboratory-generated phenotypic data critically complement epidemiologic observations. Accordingly, in a recent assessment of three influenza viruses (an avian H1N1 virus, a human isolate of H7N9, and a human isolate of H3N2v), virologic data contributed highly to the overall risk score. For evaluating the likelihood of emergence, laboratory data about transmission and sialic acid receptor binding specificity were about two-thirds and half as important as the extent of human infections, respectively. For evaluating potential consequences of emergence, disease severity, which reflects the severity of human infections as well as the severity in appropriate animal models, was most important.[619] The major limitation associated with laboratory-generated phenotypic data is that political, logistical, and regulatory factors delay receipt of clinical specimens/viral isolates in US labs and subsequent generation of virologic data.

Epidemiologic data

Three risk elements rely on epidemiologic data: human infections, disease severity (which is also informed by laboratory testing in animals), and population immunity (detection of pre-existing cross-reactive serum antibodies). The human infections element considers the number and frequency of human cases and evidence for human-to-human transmission, while the disease severity element considers the spectrum of illness observed in humans, including the age distribution of deaths. The human infections and disease severity elements are the most important elements of the likelihood and consequences components of the IRAT, respectively, because the data directly reflect the properties of the virus in humans. However, there are several challenges associated with the interpretation of epidemiological data for pandemic risk assessments. When a novel virus first emerges, extrapolating virus properties from a limited number of human cases may be difficult. In particular, disease severity is often initially over-estimated because only severe cases interact with the public health system, and serological studies to ascertain population exposure are difficult and time-consuming to carry out.

Ecological/environmental factors

Finally, two risk elements involve ecological factors, which collectively consider the global distribution of the virus in animals, the number of species that can be and are infected, and the potential extent of exposure between humans and those animal species. Other environmental information, such as the strength of the public health systems and the strength of the relationship between the public health and veterinary services sectors in countries in which the virus is circulating in animal populations, may also be considered. These elements moderately contribute to the likelihood component and minimally contribute to the consequence component of the IRAT. Importantly, these elements reflect completely different aspects of risk than the elements based on phenotypic, genetic, and epidemiologic data.

9.6.4.3.2 Public Health Impacts of Pandemic Risk Assessments: Development of Pre-Pandemic Vaccines

The development of pre-pandemic vaccines will lead to earlier vaccine availability during a pandemic, thereby reducing human morbidity and mortality, as discussed in Section 9.6.1.3.3.3. Several completely different strategies can be used to increase the availability of vaccines during a pandemic, thus achieving

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617 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

618 Trock SC *et al* (2012) Development of an influenza virologic risk assessment tool. *Avian diseases* 56: 1058-1061

619 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

the same ultimate public health goal. These strategies are described in detail in Section 9.5.4.2.2 and are briefly summarized here. First, a universal or broad-spectrum flu vaccine could be deployed in advance of a pandemic or could be rapidly deployed following the emergence of a novel pandemic strain. However, influenza and vaccinology experts disagree about the scientific feasibility of developing a universal vaccine, and one expert felt that a ten to twenty year time frame for development is optimistic. Second, several scientific and technical advancements could shorten production timelines for strain-specific vaccines, which would lead to faster vaccine availability during a pandemic. New vaccine platforms, such as recombinant vaccines, can be rapidly scaled up and have shorter production timelines than egg- and cell-based vaccines. However, the one recombinant vaccine on the market accounts for less than 1% of total seasonal influenza vaccine produced annually, and although several other virus-free vaccine platforms are in development, the length and expense of licensure processes for new vaccines will delay their widespread availability. Incorporating adjuvants into existing egg- and cell-based vaccines would allow for a smaller quantity of antigen to be used per vaccine dose, thus enabling production of the same number of doses in a shorter period of time. However, only one US-licensed pandemic vaccine includes adjuvants. Although an active area of research, adjuvanted vaccines must undergo standard FDA licensing procedures for new vaccines and thus are unlikely to be broadly available in the near future.

9.6.5 Comparison and Analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

In this section, the potential benefits of GoF research that enhances mammalian adaptation and transmissibility *relative* to alt-GoF approaches are discussed, in each benefit category that GoF approaches can address.

9.6.5.1 Benefits to Scientific Knowledge

9.6.5.1.1 Scientific Knowledge Gap 1: Can Animal Influenza Viruses Become Transmissible Between Humans?

GoF approaches are uniquely capable of *proactively* assessing the potential for *any* animal influenza viruses to acquire enhanced fitness and transmissibility in mammals. Notably, the relevance of this information for human populations depends on the suitability of animal models as well as whether laboratory-acquired mutations can arise in nature, both of which are unknown.

9.6.5.1.2 Scientific Knowledge Gap 2: How Do Animal Influenza Viruses Adapt to and Become Transmissible in Humans?

GoF approaches are uniquely capable of providing in-depth information about the evolution of mammalian fitness/transmissibility in *any* animal influenza virus strain. In addition, GoF approaches are uniquely capable of demonstrating the order(s) of acquisition of genetic changes that are necessary and sufficient to lead to enhanced fitness/transmissibility in mammals. However, the relevance of information derived from GoF approaches is contingent upon how well animal models represent human disease and how well the lab environment mimics natural evolution.

For those wild type strains that are naturally capable of productively infecting laboratory animals used for transmission studies, simply characterizing the transmissibility of a strain in animals, an alt-GoF approach, has the potential to generate similarly in-depth information. However, a single round of transmission may be insufficient for relevant adaptive changes to accrue or may reveal only part of the adaptive process, which further lessens the relative utility of this alt-GoF approach. Surveillance-based approaches, including comparison of human and animal isolates and comparison of animal isolates from different species, are uniquely capable of reporting on the real-world evolution of a variety of strains, thus

complementing two shortcomings of GoF approaches. Though results gleaned from comparative analysis of human and animal isolates are directly translatable to humans, the fact that animal influenza virus strains that efficiently transmit in humans have not been observed in nature precludes use of this approach for the study of transmissibility in particular. While case studies of interspecies transmission events exist, the translatability of that information to the evolution of human adaptive traits is uncertain.

9.6.5.1.3 Scientific Knowledge Gap 3: What Are the Genetic and Phenotypic Traits That Result in Adaption and Transmission in Humans?

GoF approaches are uniquely capable of identifying novel genetic and phenotypic traits underlying mammalian adaptation and transmissibility in *any* animal influenza virus strain of interest. Furthermore, targeted genetic modification of viruses to introduce genetic traits associated with mammalian adaptation/transmissibility is uniquely capable of demonstrating that particular genetic markers are *necessary* and *sufficient* for mammalian adaptation and transmissibility across multiple virus contexts. Given the importance of genetic context for influenza biology, this approach critically strengthens the certainty of scientific knowledge about mechanisms underlying mammalian adaptation and transmissibility. However, results gleaned from cell culture and animal model studies may not translate to human disease. Notably, attenuated strains cannot be used to study mechanisms underlying airborne transmission because these strains do not efficiently infect ferrets. Although microRNA-based strategies for "molecular biocontainment" have shown promise for transmission studies in ferrets, further research is needed to determine whether these strains will serve as reliable proxies for a wide variety of wild type viruses. In addition, miRNA-based strategies cannot be used for studies involving human cell lines, limiting their utility for *in vitro* studies examining phenotypes underlying mammalian adaptation and transmissibility.

Characterizing wild type viruses, an alt-GoF approach, also has the potential to uncover previously unknown traits. However, the fact that this approach cannot be used to study animal influenza viruses that do not productively infect laboratory animals and that relevant changes may not arise during a single round of transmission renders it less useful than GoF approaches. LoF approaches have limited utility for broad and unbiased identification of necessary genetic and phenotypic traits due to their inefficiency and the fact that mechanisms underlying transmissibility of seasonal/pandemic viruses may not translate to animal influenza viruses. The simplicity and relative high-throughput nature of *in vitro*, virus-free systems renders them appealing for the discovery of novel genetic traits that alter *known* phenotypes underlying mammalian adaptation/transmissibility, but properties observed may not be recapitulated during the complete viral life cycle.

Unlike GoF methods, the use of human and animal surveillance data for the discovery of genetic markers associated with adaptation/transmission directly translates to human disease and has strength in numbers as it analyzes genetic traits across large data sets. Critically, this approach cannot be used for studying transmissibility because animal or zoonotic viruses that efficiently transmit in humans have not been observed in nature. Analysis of sequences spanning avian to mammalian adaptation events enables the identification of "real-world" markers associated with mammalian adaptation/transmissibility but may not translate to human-adapted viruses. For both surveillance-based approaches, shortcomings in the quality and availability of surveillance data compromise the feasibility of this approach and the relevance of any findings.

Finally, host-focused approaches, such as proteomic and genomic screens, cannot supplant the identification of viral adaptation/transmissibility traits but rather complement GoF approaches by identifying host factors that contribute to those processes.

9.6.5.2 Benefits to Surveillance

A key goal of influenza surveillance is to monitor the evolution of circulating animal influenza viruses, in order to identify those viruses that pose a risk of emerging in human populations to cause a pandemic. Resources can then be dedicated to mitigating the risks that those viruses emerge and the potential consequences of an emergence event. Analysis of the phenotypic properties of individual surveillance isolates is an important aspect of pandemic risk assessments, including transmissibility and virulence in mammals. Currently, this analysis relies on the laboratory characterization of surveillance isolates and, to a lesser extent, the inspection of sequences for molecular markers associated with phenotypes underlying mammalian adaptation, transmissibility, and virulence. Both methods exhibit shortcomings that compromise the accuracy, timeliness, and quantity of data. Two additional approaches have been proposed: the development of rapid assays for phenotypes underlying mammalian adaptation and transmissibility, and the use of computational models to predict underlying phenotypes from genotype. Such rapid phenotypic assays do not yet exist, and the prospective accuracy of existing models is unknown. Both GoF and alt-GoF experimental approaches have potential to address shortcomings associated with the use of rapid phenotypic assays, molecular markers, and computational models, thereby benefitting surveillance of animal influenza viruses.

GoF approaches provide unique benefits to the design and validation of rapid assays for phenotypes underlying adaptation, transmissibility, and virulence. The fact that these assays would be high-throughput and less technically challenging than ferret experiments could increase the quantity and timeliness of phenotypic data available, relative to the use of traditional phenotypic characterization assays for adaptation, transmissibility, and virulence. The accuracy and utility of rapid phenotypic assays depends on establishing a strong linkage between underlying phenotypes and adaptation/transmissibility/virulence as well as developing an understanding of how multiple phenotypes synergize to enhance the infectivity, transmissibility, and virulence of animal influenza viruses in mammals. GoF approaches represent the most efficient and effective approach for discovering novel phenotypes underlying mammalian adaptation, transmissibility, and virulence and are uniquely capable of demonstrating that a particular phenotype is causally linked to enhanced infectivity/transmissibility/virulence in mammals across multiple virus contexts. GoF approaches are also uniquely capable of causally determining how multiple underlying phenotypes interact to enhance infectivity, transmissibility, or virulence in mammals, which provides insight into how information about underlying phenotypes should be integrated for a risk assessment. However, a major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to human disease in nature. Characterizing the constellation of underlying phenotypes present in a large number of wild type viruses (alt-GoF) is uniquely capable of providing insight into whether partially adapted viruses can persist in nature, which lends support to the practice of inferring complex phenotypes such as adaptation, transmissibility, and virulence based on data about underlying phenotypes. In addition to the need for scientific advancements, a notable barrier to realization of the benefits derived from the use of rapid phenotypic assays is that these assays must be carried out under BSL-3 conditions, which limits the number of diagnostic laboratories that will be able to conduct the assays.

GoF approaches provide unique benefits to the practice of using molecular markers to infer phenotypes underlying adaptation/transmissibility/virulence based on genetic sequence data. The fact that sequence data can be reliably generated at NICs and other diagnostic labs in developing countries can increase the timeliness and quantity of phenotypic data available, relative to the conduct of traditional phenotypic characterization assays at WHOCCs. Currently, most molecular markers for mammalian adaptation, transmissibility, and virulence have low predictive value due to significant scientific uncertainties associated with the association between underlying phenotypes and adaptation/transmissibility/virulence, whether the function of markers is conserved across different strain contexts, and incomplete knowledge about the breadth of mutations that can give rise to a particular phenotypic change. As discussed above,

GoF approaches provide essential data for strengthening the linkage between underlying phenotypes and adaptation/transmissibility/virulence. GoF approaches also provide unique advantages for discovering novel markers and strengthening the predictive value of known markers. Namely, GoF approaches represent the most efficient and effective approach for discovering novel genetic traits underlying mammalian adaptation, transmissibility, and virulence and are uniquely capable of demonstrating that particular genetic markers are *necessary* and *sufficient* for mammalian adaptation, transmissibility, or virulence across multiple virus contexts. However, the validation of molecular markers for mammalian adaptation or virulence through analysis of genetic surveillance data (alt-GoF) is uniquely capable of providing direct insight into traits associated with human adaptation/virulence across multiple strain contexts, which complements GoF approaches. Notably, surveillance-based approaches are not viable for the validation of molecular markers associated with transmissibility because animal influenza strains that transmit efficiently between humans in nature do not exist. GoF approaches are also uniquely capable of systematically exploring alternative mutational pathways for altering an underlying phenotype in the context of whole virus. *In vitro*, virus free approaches can also be used, but results may not be recapitulated in the context of the full virus. As above, the major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to human disease in nature. Finally, in addition to the need for scientific advancements, a notable barrier to the full realization of benefits derived from the use of molecular markers is the need to expand sequencing capabilities at NICs.

GoF approaches are also critical for improving models for prediction of underlying phenotypes based on sequence data. Specifically, GoF approaches that generate information about mutations that do and do not give rise to phenotypic changes of interest provide critical training data for models, and GoF approaches are needed to validate model predictions in the context of the full virus. Importantly, other types of biophysical data generated through alternative experimental approaches are also critical for improving the accuracy of existing models. Similar to the use of molecular markers, full realization of the benefits derived from the use of computational models will require significant scientific advancements as well as the expansion of sequencing capabilities at NICs.

Both the direct measurement of virus phenotypes in the laboratory and the prediction of underlying phenotypes from genotype, either through sequence inspection for molecular markers or computational modeling approaches, have inherent strengths and limitations. Namely, the generation of phenotypic data will always be delayed by the need to ship virus samples, but direct measurements of phenotypic properties are invaluable. In contrast, as sequence data is increasingly available from NICs and other "base" level diagnostic laboratories, the application of predictive methods will enable the rapid generation of phenotypic "data" that reflects the properties of viruses present in clinical samples, allowing for more rapid characterization of emerging influenza viruses. However, due to the inherent uncertainties associated with predictions, the subsequent confirmation of predictions through phenotypic testing is critical. Therefore, virological data and sequence-based predictive data are complementary, and consideration of both will strengthen the timeliness and accuracy of assessments of virus properties that contribute to pandemic risk.

9.6.5.3 Benefits to Pandemic Risk Assessment, Decision-Making in Public Health Policy

GoF approaches have potential to benefit pandemic risk assessments by strengthening the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence, which are a component of the "genomic variation" risk element considered in the assessment. The importance of this element relative to other risk elements places a qualitative "upper bound" on the potential benefits of GoF research to pandemic risk assessments. Notably, because molecular marker data are currently incorporated into pandemic risk assessments, the benefits of GoF-derived improvements to the reliability of molecular marker data could be immediate.

Epidemiological data (alt-GoF) represent the most important input to the risk assessment, for both the likelihood and consequences of emergence component of the IRAT. Laboratory data about transmissibility and virulence in appropriate animal models and receptor binding specificity also significantly contribute to the overall pandemic risk score. Genomic variation, which includes consideration of molecular marker data for mammalian adaptation, transmissibility, and virulence, is relatively less important. Given the caveats associated with epidemiological and virological data, subject matter experts involved in the pandemic risk assessment process emphasized the value of corroborating information about infectivity, transmissibility, and disease severity in humans or appropriate animal models with molecular marker data.[620] That genetic data can increase confidence in an estimate of risk adds certainty to decision-making downstream of the risk assessment, which is valuable.

Molecular marker data play a more important role in the risk assessment when a novel influenza virus first emerges in the human population. In this scenario, epidemiological data will be scant and sequence data are likely to be available before phenotypic data. As a result, the use of molecular marker data enables a rapid risk assessment of the emerging virus, so that downstream response actions can be initiated more quickly if deemed appropriate. In the event of a pandemic, such a three to four week head start on vaccine production could significantly reduce pandemic-associated morbidity and mortality. For example, researchers estimate that deployment of vaccine two weeks earlier during the 2009 H1N1 pandemic would have prevented an additional ~600,000 cases (approximately a 60% increase in the number of cases prevented), while deployment of the vaccine four weeks earlier would have prevented an additional 1.4 million cases (approximately a 135% increase in the number of cases prevented).[621]

Once the decision is made to develop a CVV, multiple strains may be available to serve as the basis for the CVV. In the event that these strains have similar epidemiological and virological characteristics, the presence and type of molecular markers for mammalian adaptation, transmissibility, and virulence can serve to differentiate between strains. This application of GoF data enables more granular decision-making than would have been possible based on other data sources alone, which is valuable because resource limitations constrain the number of CVVs that can be produced.

International surveillance for influenza is improving, especially in the wake of the 2009 pandemic, but gaps remain, particularly in certain regions of the world (e.g., parts of Africa, regions experiencing political instability, etc.). The limited breadth of available surveillance data constrains the potential benefits of using pandemic risk assessments to guide decision-making about pandemic preparedness investments. That is, experts can only evaluate and prepare for pandemics caused by strains they know about. For that reason, all stakeholders interviewed for this report, including influenza researchers, public health personnel, and USG public health policy representatives, agreed that there is a clear need to strengthen and expand influenza surveillance networks. Importantly, expanded surveillance alone is not sufficient to improve pandemic risk assessments without concomitant improvements to the tools used for pandemic risk assessments, including the use of molecular marker data. Thus, strong surveillance networks function as a co-factor that is needed for the full realization of GoF benefits to pandemic risk assessments.

As discussed in Section 9.6.3.2, GoF approaches can also benefit surveillance for animal influenza viruses by enabling the development of rapid assays for phenotypes underlying mammalian adaptation, transmissibility, and virulence, as well as by improving computational models for sequence-based predictions of underlying phenotypes. Either type of data could be used to corroborate information about

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620 (2015c) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.

621 Borse RH *et al* (2013) Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. *Emerging infectious diseases* 19: 439-448.

transmissibility and virulence gleaned through ferret experiments. Given the variability inherent in animal experiments, data about underlying phenotypes could strengthen the robustness of this phenotypic information. However, the timeline for realization of this benefit is likely to be long-term. The benefits arising from rapid phenotypic assays depends on the discovery and validation of suitable underlying phenotypes and the development and validation of an appropriate rapid phenotypic assay. The benefits arising from the use of computational models depend on the development of reliable models, which will likely prove to be a significant scientific challenge. The timescales for these scientific and technical innovations are unknown.

9.7 Influenza Viruses: Benefits of GoF Research That Enhances Virulence

9.7.1 Summary

This section describes the benefits of GoF research that is reasonably anticipated to enhance the virulence of influenza viruses in representative animal models. Such GoF studies were found to generate scientific knowledge; to inform surveillance of circulating animal influenza viruses, which has downstream impacts on decision-making about USG investments in pandemic preparedness initiatives; and to inform the development of new influenza vaccines and therapeutics. Alt-GoF approaches that may generate similar benefits were also identified and analyzed. At present, GoF studies resulting in enhanced virulence in mammals have unique benefits to scientific knowledge, surveillance, and pandemic preparedness, though full realization of GoF benefits to public health requires significant scientific advancements. Chapter 9.7 provides an overview of these benefits, including basic background and Supporting Information; a fully referenced and more thorough discussion of these benefits can be found in Appendix IV Section 15.4.

9.7.1.1 Benefits of GoF That Enhances Pathogenicity to Scientific Knowledge

- GoF approaches:
  - Are the most efficient and effective strategies for identifying novel viral genetic and phenotypic traits underlying pathogenicity and are uniquely capable of demonstrating that a particular viral trait is necessary and sufficient to enhance virulence. However, results in model systems may not translate to human disease.
  - Are capable of identifying host factors that are associated with enhanced pathogenicity.
  - Are capable of generating animal models that recapitulate human disease for the study of pathogenicity through adaptation of viruses to host animals. However, adaptive mutations may alter the biology of the virus.
- Alt-GoF approaches:
  - Are uniquely capable of providing direct insight into genetic traits associated with enhanced virulence in humans, but are severely constrained by the quality and availability of existing surveillance data.
  - Are capable of demonstrating that particular viral traits are necessary for virulence, which complements GoF approaches.
  - Are uniquely capable of confirming that a particular host factor contributes to virulence and/or deleterious host immune responses.

- Are capable of generating animal models for the study of pathogenicity and to support MCM development through sensitization of host animals to viral infection through targeted gene knockout or the use of immunosuppressants. However, results using immunocompromised animals may not translate to healthy hosts.

9.7.1.2 Benefits of GoF That Enhances Pathogenicity to Surveillance

- GoF approaches:
  - Provide a foundation for the development of rapid assays for phenotypes underlying virulence, which have potential to increase the quantity and timeliness of phenotypic information about animal flu viruses detected through surveillance. However, the success of this approach is subject to significant advancements in the state of knowledge about mechanisms underlying pathogenicity.
  - Are uniquely capable of strengthening the predictive value of molecular markers for virulence, which can be used to infer phenotype from sequence. Use of molecular markers in lieu of or to corroborate phenotypic testing results could improve the quality, timeliness, and quantity of phenotypic information about animal flu viruses detected through surveillance. However, the success of this approach is subject to significant advancements in the state of knowledge the mechanistic basis of pathogenicity, and predictions must be experimentally validated.
  - Are critical for improving computational models for predicting phenotypes underlying pathogenicity based on sequence, which could improve the quantity and timeliness of phenotypic information about animal flu viruses detected through surveillance. However, the success of this approach is subject to significant advancements in the state of knowledge the mechanistic basis of pathogenicity, and predictions must be experimentally validated.
- Alt-GoF approaches:
  - Have significant limitations for advancing the development of rapid assays for phenotypes underlying pathogenicity and for strengthening the predictive value of molecular markers for virulence.
  - Are also critical for improving computational models for predicting phenotypes underlying pathogenicity based on sequence, but through the generation of different types of data that complement data generated through GoF approaches.
  - Phenotypic assays for virulence are uniquely capable of providing direct information about this complex phenotype under controlled conditions, but results may be delayed relative to the publication of viral sequences or, in the future, the generation of data about underlying phenotypes through rapid assays.

9.7.1.3 Benefits of GoF That Enhances Pathogenicity to Decision-Making in Public Health Policy

- GoF approaches:
  - Are uniquely capable of strengthening the predictive value of molecular markers for virulence, which moderately influence pandemic risk assessments of circulating animal influenza viruses, relative to other types of data that are considered in the assessment. Pandemic risk assessments guide downstream decisions about investments in pre-pandemic vaccines, which will increase vaccine availability during a pandemic if a similar strain emerges to cause a pandemic.

- Molecular marker data plays a relatively more important role when novel influenza viruses first emerge in human populations, when epidemiological data are scarce and virological data are not yet available. The ability to conduct a rapid risk assessment using molecular marker data can provide a three to four week head start on vaccine production.

- Molecular marker data can guide selection of particular viruses to use as the basis of pre-pandemic vaccines, when multiple viruses have similar epidemiological and virologic characteristics.

- Alt-GoF approaches:
  - Epidemiological data are the most influential data in a pandemic risk assessment, but disease severity can difficult to accurately measure in human populations, and epidemiological data may be scarce when novel viruses first emerge in human populations.

  - Virologic data strongly influence pandemic risk assessments, but the generation of virological data may be delayed relative to the publication of sequencing data when novel viruses emerge abroad, due to shipping delays.

  - Other types of data, such as ecological data, also contribute to pandemic risk assessments but completely molecular marker data (GoF) by evaluating completely different aspects of pandemic potential.

9.7.1.4 Benefits of GoF That Enhances Pathogenicity to Vaccine Development

- GoF approaches:
  - Are uniquely capable of determining whether live attenuated influenza vaccine (LAIV) candidates recover virulence upon growth in cells or animals, an important aspect of safety testing. LAIVs are being explored as potential pandemic vaccines for avian influenza viruses and have shown promise.

  - Can be used to discover genetic traits that confer enhanced virulence, which can be removed from vaccine viruses to increase the safety of vaccine production. However, alt-GoF approaches must first be used to demonstrate that mutating particular virulence markers is sufficient to attenuate the virulence of vaccine viruses.

  - Are capable of generating animal models that recapitulate human disease to support vaccine development through adaptation of viruses to host animals. However, adaptive mutations may alter the susceptibility of the virus to vaccines, rendering results misrepresentative.

- Alt-GoF approaches:
  - Several alternative vaccine platforms are also being explored as potential pandemic vaccines for avian influenza viruses and have shown promise.

  - Are uniquely capable of determining that mutating or deleting particular virulence markers attenuates the virulence of vaccine viruses, which can improve the safety of vaccine production.

  - Are capable of generating animal models for the study of pathogenicity and to support vaccine development through sensitization of host animals to viral infection through targeted

gene knockout or the use of immunosuppressants. However, results using immunocompromised animals may not translate to healthy hosts.

9.7.1.5 Benefits of GoF That Enhances Pathogenicity to the Development of Therapeutics

- GoF approaches:
  - Represent the most efficient and effective strategies for identifying novel viral factors that contribute to virulence, which may be good targets for new therapeutics.
  - Are capable of generating animal models that recapitulate human disease to support therapeutic development through adaptation of viruses to host animals. However, adaptive mutations may alter the susceptibility of the virus to therapeutics, rendering results misrepresentative.
- Alt-GoF approaches:
  - Represent the most effective strategies for identifying novel host factors that contribute to virulence, which may be good targets for new therapeutics.
  - Other alternative approaches for the development of new therapeutic candidates, including high-throughput screening of small molecule compounds and selection of monoclonal antibodies that bind to particular virus proteins, are also being actively pursued and have generated promising therapeutic candidates.
  - Are capable of generating animal models for the study of pathogenicity and to support vaccine development through sensitization of host animals to viral infection through targeted gene knockout or the use of immunosuppressants. However, results using immunocompromised animals may not translate to healthy hosts.

9.7.2 Overview of GoF Research Landscape: Enhanced Pathogenicity in Representative Animal Models

9.7.2.1 Serial Passaging of Viruses in Cell Culture or Animal Models

Serial passaging of viruses in cell culture or animals selects for viruses with enhanced fitness or virulence, respectively. This approach is performed for three purposes. First, serial passaging is utilized to develop animal models for studying the mechanistic basis of flu-associated morbidity/mortality and for medical countermeasure development. Second, this approach enables the identification of mutations that are associated with enhanced fitness/virulence, which provides a foundation for follow-up studies that investigate the mechanistic basis of pathogenicity. These studies can also provide insight into host mechanisms underlying disease pathology by correlating host immune responses with morbidity and mortality measures. Third, the serial passaging approach is used to determine whether attenuated strains are capable of recovering virulence upon passage *in vitro* or *in vivo*. This third type of serial passaging study may be carried out using live attenuated influenza vaccine (LAIV) candidates, as an important aspect of safety testing prior to human clinical trials. In addition, these studies may be conducted using strains with fitness defects arising from the acquisition of antiviral resistance or other GoF phenotypes, in order to gain insight into the likelihood that these strains will persist and spread in nature. All types of serial passaging studies may be performed with seasonal or animal (i.e., avian and swine) viruses, and animals such as mice, ferrets, and swine may be used. Of note, serial passaging studies involving attenuated strains simply increase the human health risk of the attenuated strain to approach that of wild type strains.

9.7.2.2 Forward Genetic Screen to Identify Mutations That Enhance Fitness/Virulence

Forward genetic screens involve random mutagenesis of genetic regions predicted to contribute to fitness/virulence or comprehensive reassortment of parental gene segments from two viruses, followed by characterization of the fitness or virulence of mutants in appropriate mammalian model systems to select for mutant viruses with enhanced fitness/virulence. Sequencing emergent viruses enables the identification of mutations or gene segments that enhance the fitness/virulence of viruses, which provides a foundation for follow-up studies that investigate the mechanistic basis of pathogenicity in mammals. These studies are performed using human seasonal viruses, the 1918 H1N1 pandemic virus, and animal viruses. A variant of this approach involves the use of strains with impaired fitness, due to the evolution of antiviral resistance, to determine whether strains can recover fitness through the acquisition of compensatory mutations, which has been performed using seasonal strains.

9.7.2.3 Targeted Modification of Viruses to Introduce Traits That Are Expected to Enhance Fitness/Virulence in Mammals

Targeted genetic modification of viruses, namely site-directed mutagenesis and/or reassortment, to introduce genetic traits that are expected to enhance the fitness/virulence of viruses followed by characterization of the fitness/virulence of mutants in cell culture or animal model systems, respectively, may lead to the generation of viruses with enhanced fitness/virulence in mammals. This approach is performed for two purposes: (1) to determine whether a previously characterized underlying genetic or phenotypic trait, such as evasion of a particular innate immune response, contributes to the complex phenotype of pathogenicity, and (2) to confirm that a particular mutation or gene segment is necessary and sufficient to enhance the fitness/virulence of viruses in appropriate model systems. Traits that are associated with enhanced pathogenicity may be discovered through GoF approaches, such as serial passaging, or alt-GoF approaches, such as random mutagenesis followed by screening for attenuated virulence (Loss of Function). As above, this information provides a foundation for follow-up studies investigating the mechanistic basis of pathogenicity. These studies are performed using human seasonal viruses, the 1918 H1N1 pandemic virus, and animal viruses.

We note that the relationship between viral fitness and pathogenicity is complex and that many of the viral traits that contribute to fitness, either directly or indirectly, mediate pathogenicity. As a result, serial passaging of viruses in animals may select for both enhanced fitness and enhanced virulence. However, enhanced viral fitness *in vivo* does not necessarily translate to high pathogenicity, as seasonal influenza viruses do not display the morbidity and mortality displayed during infections with zoonotic influenza viruses such as H5N1, but grow to a high titer.

9.7.3 Identification of the Potential Benefits and Limitations of GoF Approaches

Here we evaluate the potential benefits of GoF research that enhances fitness and pathogenicity in each benefit category listed in the NSABB Framework.

9.7.3.1 Benefits and Limitations of GoF Approaches to Scientific Knowledge

GoF approaches have potential to benefit scientific knowledge in several ways. First, GoF approaches can provide insight into the mechanistic basis of pathogenicity, including the identification of viral and host traits that contribute to pathogenicity. Second, GoF approaches enable the identification of compensatory mutations that rescue the growth of antiviral resistant strains, which provides a foundation for follow up studies investigating the mechanistic basis of the enhanced fitness phenotype. Finally, viruses with enhanced virulence developed using GoF approaches can be used as tools to understand how the host immune response contributes to morbidity and mortality observed during influenza infections. The

benefits and limitations of GoF approaches in each of these scientific areas is addressed in more detail below.

9.7.3.1.1 Scientific Knowledge Gap 1: What Are the Viral Genetic and Phenotypic Traits That Underlie Pathogenicity in Mammals? What Are the Host Factors That Contribute to Enhanced Pathogenicity, as Well as Infection-Associated Morbidity and Mortality?

Introduction

The pathogenesis of influenza viruses reflects the complex interactions between viral and host factors and is the result of both the virus's ability to cause disease and the host's response to viral infection. While advances in research have revealed functions of specific influenza proteins and genetic traits that contribute to virulence, considerable gaps in knowledge remain about the molecular basis and the role of each underlying phenotype in in defining pathogenicity and associated disease outcomes. Moreover, there is limited understanding of the host factors that contribute to protective versus deleterious outcomes. Insight into virus-host interactions is needed to advance in-depth understanding of virulence and pathogenesis of influenza viruses.

Benefits and limitations of GoF approaches

Several GoF approaches can be used to discover the genetic and phenotypic markers underlying enhanced pathogenicity of influenza viruses:

- Targeted genetic modification to introduce novel genetic changes that are expected to contribute to pathogenicity by either site-directed mutagenesis or targeted reassortment (often between animal-origin or human pandemic and human seasonal strains),
- Forward genetic screens involving random mutagenesis or comprehensive reassortment followed by selection for enhanced virulence or underlying phenotypes, and
- Serial passaging in appropriate animal models or mammalian cells to select for viruses with enhanced pathogenicity.

Collectively, these approaches enable the identification of genetic changes that are sufficient to confer enhanced pathogenicity in representative model systems. The GoF approaches described here also provide insight into host response pathways that contribute to underlying disease pathology. Serial passaging has the potential to uncover *novel* viral genetic and phenotypic traits that contribute to enhanced virulence. In contrast, because forward genetic screens involving random mutagenesis typically focus on regions that are suspected or known to play a role in phenotypes underlying pathogenicity, this approach can discover novel viral *genetic* markers for enhanced virulence only. The targeted genetic modification approach is limited to the investigation of viral genetic traits and underlying phenotypes that are suspected to contribute to pathogenicity (e.g., determining whether enhanced polymerase activity contributes to pathogenicity).

Targeted genetic modification is also used to confirm that particular virus mutations or gene segments are *necessary* and *sufficient* to enhance virulence in mammals. Often this experiment is followed by characterization of other virus phenotypes, such as infectivity and tissue tropism. Furthermore, this approach provides associative insight into how host responses are altered during infection with the modified strain. Collectively, this information provides a strong foundation for follow-up studies investigating the mechanistic basis of pathogenicity, including the study of host-virus interactions.

Taken together, these GoF studies provide a foundation for follow-up cell biological, immunological, and pathological studies that elucidate the mechanistic basis of viral factors contributing to virulence, corresponding host responses, and how both factors alter susceptibility to secondary bacterial infection. Additionally, GoF approaches permit the identification of host immune responses that are associated with enhanced pathogenicity. Although the analysis of host factors contributing to enhanced pathogenicity is indirect, this information can be derived from the comparison of genetically similar virus backgrounds displaying a dynamic range of virulence (i.e., GoF and parental strains). The relevance of these approaches depends on whether mechanisms underlying enhanced virulence in cell culture and animal models are representative of those in humans. Another drawback of these approaches is that results gleaned from the study of one or a few strains may not be recapitulated in different genetic contexts.

9.7.3.1.2 Scientific Knowledge Gap 2: Provide Insight into Whether Fitness Defects Associated with the Acquisition of Antiviral Resistance Can Be Overcome and the Mechanisms Underlying Recovery of Fitness

Introduction

Though influenza viruses can readily mutate to acquire resistance to therapeutics, antiviral-resistant viruses are often initially less fit than parental viruses. The relative fitness of antiviral-resistant strains has implications for how likely and how quickly these strains are to spread in nature. Whether and how antiviral strains can acquire compensatory mutations that enhance fitness while preserving the antiviral resistance phenotype is unknown for most antiviral resistance mutations. Studies investigating this question provide insight into the mechanistic basis of viral fitness and the mechanistic interplay between antiviral resistance and other virus phenotypes.

Benefits and limitations of GoF approaches

Several GoF approaches can be used to determine whether antiviral-resistance strains with impaired growth can recover fitness and to identify compensatory mutations that rescue growth, which provides a foundation for follow-up biochemical and cell biological studies that investigate the mechanistic basis of enhanced growth. First, growth-impaired strains can be serially passaged in cells or animals to select for strains with enhanced fitness, following by sequencing of emergent viruses to identify genetic changes that arose. However, this approach often results in reversion of antiviral-resistance mutations rather than the evolution of compensatory mutations. A second approach involves forward genetic screens to identify mutations that are sufficient to rescue fitness. While this approach is more likely to uncover compensatory mutations than serial passaging, screening large libraries of mutants is relatively labor-intensive, particularly if mutations are introduced into multiple virus proteins (as compensatory mutations may arise in proteins that do not contain antiviral-resistance mutations). Finally, targeted mutagenesis is used to confirm that a particular mutation or set of mutations is necessary and sufficient to rescue the fitness of a growth-impaired strain.

9.7.3.1.3 Scientific Knowledge Benefit 3: Generation of Animal Models for the Study of Flu-Associated Morbidity/Mortality and for Vaccine and Therapeutic Development

Model systems that can be efficiently infected by influenza viruses and exhibit the spectrum of disease observed during human infections are essential for the study of influenza-associated morbidity/mortality. Serial passaging of influenza viruses in laboratory animals, which enhances the virulence of the virus in that animal, generates animal models that can be used to study the mechanisms underlying the pathogenesis of influenza viruses. This approach is performed for two purposes: (1) to generate viruses capable of efficiently infecting mice, as mice are inherently resistant to infection with human seasonal

influenza viruses and some animal influenza viruses and (2) to generate viruses with enhanced pathogenicity, if wild type viruses exhibit a limited spectrum of disease in representative animal models. In particular, the generation of mouse models is useful for pathogenesis studies due to the wide array of immunological and other experimental tools that have been developed for mice. However, the passaging needed to adapt the virus to representative animal models may alter the biology of the virus, such that results do not translate to natural disease.

9.7.3.2 Benefits and Limitations of GoF approaches to Surveillance

GoF approaches that lead to the identification of molecular markers for enhanced pathogenicity have the potential to inform the interpretation of wildlife, agricultural animal, and public health surveillance information. Specifically, determining the presence (or absence) of particular mutations associated with enhanced virulence is one aspect of evaluating the risk posed by circulating animal influenza viruses, as viral virulence plays a key role in the expected public health consequences caused by a novel influenza virus emerging in human populations. The strategies for monitoring the virulence of animal influenza viruses detected through surveillance are similar to those for monitoring mammalian adaptation and transmissibility; GoF approaches that enhance virulence and those that enhance infectivity and transmissibility in representative animal models benefit surveillance through similar mechanisms. Thus, these benefits are discussed collectively in Section 9.6.3.2.

GoF approaches that lead to the identification of compensatory mutations that rescue the fitness of antiviral-resistant strains with impaired growth do not benefit surveillance. Because of the high mutation rate of influenza viruses, influenza surveillance experts expect that antiviral resistant strains that initially exhibit impaired fitness can readily acquire compensatory mutations that rescue growth. Thus, experts simply track the presence of antiviral resistance markers, and the additional presence or absence of a known compensatory mutation does not increase or decrease the level of risk associated with the antiviral resistance marker.

9.7.3.3 Benefits and Limitations of GoF to the Development of Vaccines

GoF approaches have potential to benefit the development of vaccines in three ways:

- Serial passaging of candidate live attenuated vaccine strains in animals is used to test whether strains recover virulence upon growth *in vivo*, which is an important aspect of vaccine safety.
- GoF approaches enable the identification of conserved virulence determinants in the HA and NA proteins. These markers may be removed vaccine viruses through targeted deletion or mutagenesis, as is commonly done for the multi-basic cleavage site present in the HA proteins from some avian influenza strains, which may improve the efficacy and safety of the vaccine production process.
- Animal models developed using GoF approaches can be used for testing the safety and efficacy of vaccine candidates.

9.7.3.3.1 Vaccine Development Benefit 1: Development of New Influenza Vaccine Candidates

Introduction – current strategies and challenges for developing pandemic vaccines for avian influenza viruses

Standard methods for production of seasonal influenza vaccines have posed challenges for the production of vaccines targeting highly pathogenic avian influenza strains such as H5N1.[622] In addition, egg-based production systems are not amenable to rapid scale-up due to their reliance on the egg supply, which would pose a major problem if a novel pandemic virus emerged off production cycle. For these reasons, researchers are exploring a variety of other platforms for the production of vaccines for avian influenza viruses with pandemic potential. Live attenuated influenza vaccines (LAIVs) are attractive for pandemic vaccines for several reasons related to their efficacy and relative ease of production and administration.[623] However, a major concern associated with LAIVs is their potential to regain virulence in people, through reversion or the acquisition of compensatory mutations.[624]

Potential benefits and limitations of GoF approaches: LAIV safety

Because of the concern that LAIVs could regain virulence in people, the WHO recommends serial passaging of LAIV candidates during the non-clinical phase of *in vivo* toxicity and safety testing (a GoF approach), to determine whether the LAIV is genetically stable or recovers virulence upon passage in animals.[625, 626] In accordance with these recommendations, multiple candidate LAIVs have been subjected to serial passaging in animals.[627,628,629, 630]

9.7.3.3.2 Vaccine Development Benefit 2: Targeted Mutagenesis to Remove Virulence Markers from Vaccine Viruses

Background – challenges for production of vaccines for highly pathogenic avian influenza viruses

Removal of the multi-based cleavage site from the HA protein of highly pathogenic avian influenza (HPAI) strains, a major determinant of viral virulence, is standard practice for the production of HPAI vaccines.[631] This mutagenesis further attenuates the vaccine virus (which is also attenuated through reassortment with an attenuated vaccine backbone strain such as PR8), enabling safe and efficient production of vaccine in eggs (or cells) under BSL-2 conditions. In the future, other conserved determinants of virulence in the HA and NA proteins of avian influenza (AI) viruses could be similarly deleted from AI vaccine viruses in order to further improve the safety of the vaccine production process.

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622 Baz M *et al* (2013) H5N1 vaccines in humans. *Virus Res* 178: 78-98
623 Ibid.
624 Ibid.
625 WHO Expert Committee on Biological Standardization. (2010) Recommendations to assure the quality, safety and efficacy of influenza vaccines (human, live attenuated) for intranasal administration. *WHO Technical Report Series No 977, 2013*. The World Health Organization., Geneva, Switzerland pp. 163-196.
626 The World Health Organization. (2005) WHO guidelines on nonclinical evaluation of vaccines. *WHO Technical Report Series, No 927, 2005*, Geneva, Switzerland, pp. 32-63.
627 Jang YH, Seong BL (2012) Principles underlying rational design of live attenuated influenza vaccines. *Clinical and experimental vaccine research* 1: 35-49
628 Han P-F *et al* (2015) H5N1 influenza A virus with K193E and G225E double mutations in haemagglutinin is attenuated and immunogenic in mice. *Journal of General Virology* 96: 2522-2530
629 Baz M *et al* (2013) H5N1 vaccines in humans. *Virus Res* 178: 78-98
630 Sedova ES *et al* (2012) Recombinant influenza vaccines. *Acta Naturae* 4: 17-27
631 (2015e) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

Potential benefits and limitations of GoF approaches

As discussed above (Section 9.7.3.1.1), GoF approaches, in particular forward genetic screens and serial passaging, represent efficient and effective methods for discovering novel viral genetic and phenotypic traits that contribute to virulence. This information provides a foundation for follow-up LoF studies that aim to determine how to *attenuate* virulence, the goal of vaccine virus development, through mutation or deletion of those traits.

9.7.3.4 Benefits and Limitations of GoF to the Development of Therapeutics

GoF approaches have potential to benefit the development of influenza therapeutics in two ways:

- GoF approaches that provide insight into viral and host traits that contribute to virulence identify potential targets for next-generation therapeutics (either targeting the virus or the host), and
- Animal models developed using GoF approaches can be used for testing the safety and efficacy of therapeutic candidates.

9.7.3.4.1 Therapeutic Development Benefit: Inform the Development of Next-Generation Therapeutics

Only one class of licensed antivirals is recommended for use in the US, the neuraminidase inhibitors (NAIs).[632] Mutations that confer resistance to one or multiple NAIs have been observed in nature, though are not yet widespread, and the NAIs exhibit limited efficacy.[633] Thus, there is an urgent need for the development of new therapeutics against influenza viruses.[634] Researchers are actively working to develop next-generation influenza therapeutics that directly target viral proteins as well as therapeutics that inhibit host factors that are critical for viral virulence or that exacerbate infection-associated pathology. GoF approaches have potential to benefit the development of both types of therapeutics.

9.7.3.4.2 Potential Benefits of GoF to Therapeutic Development

As discussed in detail in Section 9.7.3.1.1, GoF approaches represent the most efficient and effective strategies for discovering novel viral genetic traits that contribute to pathogenicity, which may be good targets for novel therapeutics. In addition, targeted genetic modification of viruses to introduce traits associated with pathogenicity is uniquely capable of demonstrating that particular viral genetic traits are *necessary* and *sufficient* to enhance virulence across multiple virus contexts, which provides a strong mechanistic basis for the role of that viral factor in virulence.

GoF approaches also enable the identification of host factors that are *associated* with virulence and immunopathology, which may be good targets for novel host-targeted therapeutics. However, because the GoF approach is indirect, the role of a particular host protein in virulence/immunopathology must be confirmed using alt-GoF approaches, which provides an important conceptual foundation for the design of therapeutics targeting that protein. Nonetheless, targeted modification to introduce mutations that are expected to enhance pathogenicity (GoF) provides a controlled system for studying the interplay between virus and host factors that contribute to pathogenicity, which is a valuable complement to alt-GoF approaches that perturb the function of host factors, a more blunt approach. Notably, in both cases, whether inhibiting viral or host factors discovered through GoF approaches is sufficient to attenuate viral replication or infection-associated pathology must be empirically determined using alt-GoF approaches.

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632 CDC. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Update November 3, 2015. Accessed November 28, 2015.

633 Ibid.

634 (2015I) Interviews with influenza researchers.

9.7.3.5 Benefits and Limitations of GoF to Both Vaccine and Therapeutic Development: Enable the Development of MCMs

9.7.3.5.1 Background– Shortcomings in Existing Influenza Vaccines and Therapeutics

Shortcomings in existing influenza vaccines and therapeutics compromise public health preparedness for influenza pandemics and exacerbate the public health consequences of annual influenza epidemics, highlighting the need for development of new influenza vaccines and therapeutics. Testing the safety and efficacy of candidate MCMs in animal models is a critical aspect of MCM development. Mice, a common animal model used for the development of influenza MCMs, are naturally resistant to infection with many influenza viruses. GoF or alt-GoF approaches can be used to develop animal models to study the effectiveness of MCMs against these viruses. The development of MCMs that protect against severe disease necessitates testing the efficacy of candidate MCMs in animal models that exhibit exacerbated disease pathology. In cases where wild type viruses cause a limited spectrum of disease, GoF or alt-GoF approaches may be used to generate model systems that display a larger dynamic range of virulence.

9.7.3.5.2 Potential Benefits and Limitations of GoF Approaches: Determining MCM Safety and Efficacy

GoF approaches to generate new model systems for characterizing the safety and efficacy of MCMs involve serial passaging of viruses in animals to enhance the infectivity and virulence of the virus toward that host. Two variants of this approach support MCM development. First, as mice are naturally resistant to many influenza viruses, passaging of those viruses in mice generates a model system for testing the efficacy of MCMs against that virus. Second, passaging of virus in ferrets to enhance virulence generates a model system exhibiting exacerbated pathology, which can be used to screen MCM candidates for their ability to protect against severe disease.[635] One key strength of this approach is that comparing the efficacy of MCMs following challenge with two genetically similar viruses provides certainty that differences in outcomes reflect true distinctions between the function of MCMs rather than disparate interactions with genetically different viruses. The main drawback associated with these approaches is that the changes that accrue during passaging may alter the susceptibility of the virus to the MCM under study, thus compromising the relevance of any results.

9.7.3.6 Benefits and Limitations of GoF to Diagnostics

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[636]

9.7.3.7 Benefits and Limitations of GoF to Inform Policy Decisions

GoF approaches that lead to the identification of molecular markers for enhanced pathogenicity contribute to assessments of the pandemic risk posed by circulating animal influenza viruses, which are based on genetic surveillance data and several other types of data (e.g., epidemiologic data, phenotypic data, etc.). These assessments inform policy decisions related to public health preparedness for novel influenza outbreaks, including whether to develop pre-pandemic vaccines. This GoF benefit to decision-making in public health policy is discussed in detail in Section 9.6.3.3.2, as evaluation of the transmissibility of animal influenza viruses similarly informs pandemic risk assessments and downstream decision-making.

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[635] Ibid.

[636] New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

9.7.3.8 Economic Benefits

GoF benefits to the development of new vaccines and therapeutics could have downstream economic benefits. We did not explicitly evaluate economic benefits in this report.

9.7.4 Identification of the Potential Benefits and Limitations of Alt-GoF Approaches That Provide Similar Potential Benefits to the GoF Approaches Being Examined

In this section, an overview of alternative (alt-GoF) approaches that yield the same or similar benefits as the GoF approaches described above is provided. Two types of alt-GoF approaches are reviewed: (1) alternative experimental approaches that can provide the same or similar scientific information as GoF experimental approaches and (2) alternative scientific and technical innovations that can yield the same public health benefits as GoF approaches, but through different mechanisms. For each approach, the scientific outcomes of the approach and how that information leads to similar benefits as GoF approaches are described.

9.7.4.1 Potential Benefits and Limitations of Alt-GoF to Scientific Knowledge

9.7.4.1.1 Scientific Knowledge Gap 1: What Are the Viral Genetic and Phenotypic Traits That Underlie Pathogenicity in Mammals? What Are the Host Factors That Contribute to Enhanced Pathogenicity, as Well as Infection-Associated Morbidity and Mortality??

Several alt-GoF approaches can be used to uncover genetic and phenotypic traits underlying pathogenicity in mammals. First, comparing the sequences of human isolates that display varying degrees of pathogenicity enables the identification of genetic changes that are associated with increased virulence. Unlike the GoF approaches described above, this approach has the potential to directly identify genetic traits that contribute to pathogenicity in humans and may be more likely to uncover conserved traits through analysis of a large number of strains. However, this approach is subject to significant limitations relative to GoF approaches. First, the utility of this approach is significantly constrained by the quality and availability of existing surveillance data. Second, the use of consensus sequences in standard surveillance practices may not be able to uncover genetic traits that are present at low frequencies in human populations. Finally, the extensive genetic diversity within circulating virus populations makes discerning distinct viral genetic traits that are likely to contribute to pathogenicity difficult, which practically limits this approach to the investigation of traits or regions previously known to be important for pathogenicity.

A variant of the surveillance-based approach involves corroboration of sequence data with immunopathological observations from autopsies, which provides an opportunity to identify host factors or genetic polymorphisms that are broadly associated with severe disease.[637] In addition to the limitations described above, this approach is limited by the availability of autopsy data and is subject to the caveat that autopsies represent late stage, lethal disease, which may not be representative.

Comparing the sequences of isolates within patients, over the course of infection and/or from different tissue sources, represents another approach for identifying genetic traits that contribute to pathogenicity in humans. Specifically, comparing early and late isolates during prolonged disease and comparing isolates from the primary site of infection (i.e., the upper respiratory tract) and those from disseminated sites (i.e., lower respiratory tract), which are associated with increased virulence, enables the identification of adaptive mutations that enhance virulence. A strength of this approach is that the reduced viral genetic diversity observed within a single patient may enable the identification of novel genetic traits associated

[637] Everitt AR *et al* (2012) IFITM3 restricts the morbidity and mortality associated with influenza. *Nature* 484: 519-523

with virulence. However, this approach is limited to the analysis of viral isolates from patients presenting with severe disease, which may not be representative.

Phenotypic characterization of wild type viruses in appropriate cell culture or animal models is another alt-GoF approach that can be used to study mechanisms underlying pathogenicity in mammals. Specifically, comparing the sequences of wild type viruses with varied levels of fitness *in vitro* and pathogenicity *in vivo* enables the identification of genetic and phenotypic traits associated with increased virulence. Similar to GoF approaches, this approach can also identify host response pathways that are associated with varying disease outcomes, including susceptibility to secondary infection. Because of the high genetic diversity among existing viral isolates, phenotypic characterization is often limited to the analysis of known determinants of pathogenicity unless highly genetically similar strains are available. Additionally, the use of *in vivo* models is restricted to the study of viruses that can productively infect representative animal model systems, which excludes some animal-origin viruses with low fitness. (Such strains are typically passaged in mice for adaptation prior to analysis of virulence, which represents a GoF approach.) As for the GoF approaches, genetic and phenotypic traits uncovered through this approach may not translate to humans.

Loss of Function (LoF) approaches, genetic screens that utilize random mutagenesis or targeted genetic modification to identify changes that attenuate fitness/virulence, can also provide information about genetic and phenotypic traits that contribute to pathogenicity. The screening approach has the potential to identify novel genetic traits associated with pathogenicity, while the targeted approach is used to confirm whether particular genetic traits are *necessary* for pathogenicity. This information complements that generated by GoF methods, but LoF approaches suffer from several limitations. First, because of the high mutation rate of influenza viruses, LoF mutations that attenuate pathogenicity may revert during the single round of passage that is needed to characterize the virulence of the mutants (which represents a selection step). Second, although in principle LoF screens for mutations that attenuate virulence can be performed in an unbiased manner, characterizing the pathogenicity of a large panel of mutants in animals is labor-intensive and expensive. As a result, the use of this method may be practically limited to cell culture systems or the investigation viral phenotypes previously shown to be associated with pathogenicity. Third, because many mutations attenuate pathogenicity for trivial reasons, for example mutations that compromise viability, discovering traits that directly contribute to virulence in high pathogenicity strains relative to low pathogenicity strains may be difficult using a LoF approach.

The use of replication incompetent viruses provides another alternative method for the identification of genetic and phenotypic traits underlying pathogenicity.[638] In these model systems, viral replication and immune evasion pathways, both of which contribute to pathogenicity *in vivo*, can be assessed in cell culture lines that are engineered to stably express an essential viral protein that is missing from the "replication-incompetent" virus strains used for infection. The result is a virus that is biologically constrained to replication in that cell line, which therefore poses low risk to people.[639,640] Using these systems, viruses can be serially passaged to identify novel adaptive mutations that are associated with phenotypes underlying pathogenicity. However, cell culture systems cannot provide information about the effect of identified genetic traits on global host responses, virus dissemination, and associated morbidity and mortality. Additionally, *in vitro* results may not be recapitulated during *in vivo* infection.

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[638] The use of this approach has been proposed during interviews with influenza researchers as a possible method, although the use of this approach for explicitly identifying genetic and phenotypic viral and host factors contributing to fitness and cell-specific immune evasion is currently limited.

[639] Martínez-Sobrido L *et al* (2010) Hemagglutinin-Pseudotyped Green Fluorescent Protein-Expressing Influenza Viruses for the Detection of Influenza Virus Neutralizing Antibodies. *Journal of virology* 84: 2157-2163

[640] Rimmelzwaan GF *et al* (2011) Use of GFP-expressing influenza viruses for the detection of influenza virus A/H5N1 neutralizing antibodies. *Vaccine* 29: 3424-3430

Several *in vitro* virus-free methods can be used to investigate phenotypes underlying pathogenicity. Cell biological assays (e.g., measuring viral polymerase activity) and crystallographic resolution of the structures of viral protein interactions with other viral or host factors (e.g., virus-host protein-protein complexes) can provide insight into the mechanistic and biophysical basis of underlying phenotypes. Comparative sequence analysis of viral proteins with different phenotypic properties can then enable the identification of mutations that are associated with relevant phenotypic changes or provide insight into the molecular basis for virus-host interactions. Alternatively, forward genetic screens can be used to identify novel genetic traits that contribute to underlying phenotypes, while targeted modification of viral gene segments in isolation confirms the set of genetic changes that are necessary and sufficient to alter an underlying phenotype. Though the simplicity and relatively high-throughput nature of these methods renders them appealing as a screening approach for the discovery of novel genetic traits associated with pathogenicity, these approaches are inherently limited to the investigation of previously identified viral phenotypes. An additional drawback is that results gleaned from studying the behavior of a viral protein or phenotype in isolation may not be recapitulated in the context of the full virus or *in vivo*. Although fairly rapid phenotypic assays have been developed for the study of phenotypic traits known to be associated with pathogenicity, assays to study certain phenotypic traits may be unreliable or unavailable for future phenotypes of interest.

The use of *in silico* approaches to model the biophysical properties of viral proteins, virus-host, and virus-virus protein complexes can be used to evaluate mutations that may alter phenotypes underlying pathogenicity. Although this approach may provide insight into the biophysical basis of interactions underlying phenotypes of interest, the success of the approach is limited by the accuracy of existing models.

Finally, because pathogenicity reflects virus-host interactions, several alt-GoF approaches focus on identifying and characterizing host factors that are associated with pathogenicity, which may provide indirect insight into viral mechanisms underlying virulence in representative animal models. The use of transcriptional (e.g., qRT-PCR, microarray) and translational (e.g., ELISA) expression profiling, as well as immunophenotyping (e.g., identifying the type and kinetics of immune cell recruitment) and histopathology, independently or in the context of the GoF and alt-GoF approaches discussed above, can identify host response pathways that change during infection and thus may play a role in pathogenicity. Another host-focused approach involves *in vitro* proteomic (e.g., mass spectrometry) and genomic screens (e.g., RNAi screen) utilizing both virus-free and cell culture-based infection systems to discover host factors that interact with virus proteins of interest or that are critical for underlying phenotypes such as viral replication and immune evasion. These approaches provide direct insight into host factors involved in viral fitness. However, screens may identify host proteins that are not be functionally relevant or may play minor roles in the viral life cycle *in vivo*. Following the discovery of host factors or signaling pathways that may play a role in pathogenesis, genetically modified mouse lines (e.g., knockout mice) or pharmacological inhibitors can be used to confirm the role of a particular protein, signaling pathway, or immune cell type in pathogenicity. Taken together, the strength of these approaches is that they provide direct information about host factors involved in pathogenicity. However, given the complexity of the immune response to influenza virus infection, resolving the function of particular host proteins in the context of globally altered host factors and regulatory networks may be difficult.

A second type of alternative approach involves the use of attenuated viruses, as a risk mitigation strategy. Four types of attenuated viruses could be used for such studies: (1) reassortants with surface protein gene segments from seasonal influenza viruses, to which the general population has pre-existing immunity, (2) reassortants with lab-adapted viruses (e.g., PR8), (3) strains which have virulence factors altered or deleted (e.g., deletion of the multi-basic cleavage site in HPAI HA sequences), and (4) strains which have incorporated binding sites for microRNAs (miRNAs) that are expressed in humans but not an animal model of interest, and therefore are replication-competent in experimental animals but not humans

(termed "molecular biocontainment").[641] The use of reassortants with lab-adapted strains to identify viral determinants that are *necessary* and *sufficient* to enhance virulence in a low-pathogenicity background is possible, as many of these strains are well characterized and provide a large dynamic range for evaluating increases in virulence. Despite those advantages, the results gleaned through use of the first three types of attenuated viruses are subject to the caveat of epistasis. That is, because complex, multi-genic traits depend on genetic context, causative genetic and phenotypic traits that contribute to enhanced virulence in attenuated strains may not be recapitulated in the context of other wild type strains and interactions with other factors (not present in the attenuated strain) may contribute to virulence. Similarly, differences in disease pathogenesis relative to wild type viruses further compromise the relevance of results gained through the use of some attenuated strains, in particular if the mechanism of attenuation alters phenotypes underlying virulence. Finally, although the microRNA-based molecular biocontainment strategy is considered promising by the influenza research community, only one such strategy has been developed to date, which generates strains that permit replication in ferrets but restrict replication in humans and mice. As mice and human-derived cell lines are important model systems for the study of mechanisms underlying pathogenicity, existing miRNA-based risk mitigation strategies are of limited utility for these studies. Of note, the identification of suitable miRNAs that are expressed in humans but not mice may permit the use of this strategy to conduct GoF studies that enhance virulence in mice in the future, thereby improving its broad utility.

9.7.4.1.2 Scientific Knowledge Gap 2: Discover Whether Fitness Defects Associated with the Acquisition of Antiviral Resistance Can Be Overcome, and the Mechanisms Underlying Recovery of Fitness

Two alt-GoF approaches can be used to identify compensatory mutations that may rescue the growth of antiviral-resistant strains with impaired fitness. First, comparative analysis of the sequences of antiviral-resistant strains with varying levels of fitness may enable the identification of mutations that are *associated* with enhanced fitness. However, due to the high genetic diversity among influenza viruses, generating strong hypotheses about mutations that are linked to the recovery of fitness is difficult. In addition, this approach is reactive, limited to the discovery of compensatory mutations after antiviral-resistant strains have recovered growth in nature. A second approach involves computational modeling to predict mutations that may rescue the fitness of growth-impaired strains. However, all predictions must be experimentally confirmed using targeted mutagenesis, a GoF approach. Additionally, because existing computational models cannot predict epistasis effects, the *in silico* approach is limited to the discovery of compensatory mutations that arise in the same protein carrying the antiviral-resistance mutations.

9.7.4.1.3 Scientific Benefit 3: Generation of Animal Models for the Study of Flu-Associated Morbidity/Mortality and for Vaccine and Therapeutic Development

Alt-GoF approaches to develop animal models for the study of influenza pathogenesis involve increasing host susceptibility to infection through the use of inbred mouse lines, knockout/transgenic mice, or the treatment of mice with immunosuppressants. This approach can enable the study of wild type viruses that do not efficiently infect wild type mice. The use of genetic modification is largely limited to the use of the mouse model system, for which there are a broad array of well-established tools. However, the mouse model is less representative of human disease than other animal models, such as the ferret. The use of immunosuppressants is a promising alternative. The key limitation of this approach is that results gleaned through the use of immunocompromised hosts may not translate to healthy human populations.

[641] Langlois RA *et al* (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844-847

9.7.4.2 Benefits and Limitations of Alt-GoF Approaches to Surveillance

Circulating animal influenza viruses detected through surveillance of humans and animals are monitored for their potential infectivity, transmissibility, and virulence in human populations, as these properties inform the likelihood that viruses will evolve to efficiently infect and transmit in humans and the expected public health consequences of their emergence in human populations. The strategies for monitoring the virulence of viruses detected through surveillance are similar to those for monitoring mammalian adaptation and transmissibility, and include strategies that are informed by GoF approaches and those that are independent of GoF. The latter set of approaches is discussed in Section 9.6.4.2.

9.7.4.3 Benefits and Limitations of Alt-GoF to the Development of Vaccines

9.7.4.3.1 Vaccine Development Benefit 1: Development of New Influenza Vaccine Candidates

Due to shortcomings in existing methods for influenza vaccine production, researchers are exploring a variety of other platforms for the production of vaccines for avian influenza viruses with pandemic potential.[642] While GoF approaches inform the development of LAIVs, several alternative vaccine platforms which do not rely on GoF for their development, such as recombinant vaccines, are also being explored. These vaccine platforms have strengths and limitations relative to LAIVs. For example, adjuvanted, inactivated vaccines may provide broad-spectrum immunity but require multiple doses to confer high levels of protection.[643]

9.7.4.3.2 Vaccine Development Benefit 2: Establish LAIV Safety

GoF approaches are used to demonstrate that candidate LAIVs do not recover virulence upon growth in cells or animals, an important aspect of safety testing. There are no alternative approaches that can provide similar information.

9.7.4.3.3 Vaccine Development Benefit 3: Targeted Mutagenesis to Remove Virulence Markers From Vaccine Viruses

The HA multibasic cleavage site is removed from vaccine viruses based on HPAI strains to enable their propagation in eggs and to improve the safety of the vaccine production process. Deletion of other conserved determinants of virulence in the HA and NA proteins of avian influenza (AI) viruses could further improve the safety of the vaccine production process in the future.

Several alt-GoF approaches can be used to discover novel virulence factors, including comparative analysis of surveillance data, comparative analysis of the sequences of wild type viruses with varying levels of virulence, use of replication incompetent viruses, and LoF forward genetic screens. As discussed above, each of these approaches has critical limitations for the discovery of novel virulence traits relative to GoF approaches. However, following the identification of novel genetic traits that contribute to virulence, targeted mutagenesis can be used to identify particular mutations within that genetic region that lead to attenuated virulence in multiple virus strains, which is essential for application of the information to the vaccine development process.

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642 Baz M *et al* (2013) H5N1 vaccines in humans. *Virus Res* 178: 78-98
643 Ibid.

9.7.4.4 Benefits and Limitations of Alt-GoF to the Development of Therapeutics

GoF approaches have potential to inform the development of new candidate therapeutics for influenza viruses. Several alt-GoF approaches, described below, also have potential to inform the development of new influenza therapeutics.

As discussed in detail in Section 9.7.4.1.1, alt-GoF approaches have significant limitations for the discovery of novel *viral* genetic traits and factors that contribute to virulence. However, alt-GoF approaches play a critical role in establishing the function of putative virulence traits, which complements mechanistic information that can be gleaned through GoF approaches. In particular, targeted LoF can be used to confirm that blocking or attenuating the function of a particular virulence factor is sufficient to attenuate viral replication and/or infection-associated pathology. This information establishes an evidence base for efforts to design therapeutics targeting that virulence factor.

Alt-GoF approaches provide valuable insight into host factors that enhance pathogenicity and contribute to deleterious immune responses. Specifically, the use of targeted knockout animals or pharmacological inhibition of the host factor during infection is uniquely capable of confirming that a host factor contributes to virulence and pathogenicity. Other alt-GoF approaches may be used to gain further mechanistic insight into the role of the host factor during infection, including characterization of host immune responses to identify host genes that are up-regulated during infection and LoF targeted genetic modification of viruses to tease apart the role of particular virus-host interactions in pathogenesis.[644] Taken together, these studies provide a conceptual foundation for the design of therapeutics targeting that protein.

In addition to designing therapeutics targeting specific virulence factors or pathways (virus or host), several alternative strategies are used to develop novel candidate therapeutics. One alternative approach for designing new therapeutics involves high-throughput screening of small molecule compounds to identify compounds that reduce viral replication *in vitro*, which may identify candidate therapeutics that target viral or host proteins.[645,646] This approach has generated promising candidates, including therapeutics that are in Phase III clinical trials in the US.[647] One drawback of this approach is that it is limited to the identification of compounds that reduce viral replication, which is only one aspect of virulence.

Another alternative approach involves identifying neutralizing monoclonal antibodies (mAbs) targeting virus proteins. These approaches isolating mAbs that bind to particular virus proteins, such as the HA protein, the nucleoprotein (NP), the NA protein, and the M2 protein from the B cells of convalescent

644 Cheung CY *et al* (2002) Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 360: 1831-1837

645 Furuta Y *et al* (2002) In vitro and in vivo activities of anti-influenza virus compound T-705. *Antimicrobial agents and chemotherapy* 46: 977-981

646 An L *et al* (2014) Screening and identification of inhibitors against influenza A virus from a US drug collection of 1280 drugs. *Antiviral research* 109: 54-63

647 Toyama Chemical Company, Ltd. Pipeline. https://www.toyama-chemical.co.jp/en/rd/pipeline/index.html. Last Update Accessed November 8, 2015.

patients or of mice that have been injected with the virus protein of interest.[648,649,650,651,652] Subsequently, the ability of mAbs to neutralize virus activity is tested. This approach has also generated promising therapeutic candidates, including therapeutics that have entered Phase I clinical trials.[653,654] However, mAb-based therapeutics have several drawbacks, including high production costs and the need for injection-based delivery.[655]

9.7.4.5 Benefits and Limitations of Alt-GoF to Both Vaccine and Therapeutic Development: Enable the Development of MCMs

GoF approaches have potential to benefit the development of new influenza vaccine and therapeutics by enabling the development of animal models that can be used to test the safety and efficacy of MCM candidates. Alt-GoF approaches, described below, can also be used to development animal models that support MCM development.

Alternative approaches for the development of new model systems involving sensitizing the host to infection through targeted genetic modification (use of inbred mouse lines or knockout/transgenic mice) or the use of immunosuppressants (in ferrets or mice).[656,657,658,659] A strength of this approach is that the generation of genetically similar hosts (or genetically identical hosts if immunosuppressants are used) that display a range of disease outcomes provides a controlled system for comparing the effectiveness of MCM candidates to protect against more severe disease. The use of genetic modification is largely limited to the use of the mouse model system, for which there are a broad array of well-established tools. However, the mouse model is less representative of human disease than other animal models, such as the ferret. The use of immunosuppressants is a promising alternative. The key drawback of this approach is that results gleaned from the use of immunocompromised hosts may not translate to disease in healthy hosts.

The infection of wild type hosts with wild type viruses represents another alternative approach, which is more relevant to human disease than other model systems. However, the utility of this approach for the mouse model system is limited because mice are naturally resistant to infection with many wild type influenza viruses. For the use of the ferret model system, wild type viruses may display a limited range of

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648 Krause JC *et al* (2011a) A broadly neutralizing human monoclonal antibody that recognizes a conserved, novel epitope on the globular head of the influenza H1N1 virus hemagglutinin. *Journal of virology* 85: 10905-10908

649 Clementi N *et al* (2011) A human monoclonal antibody with neutralizing activity against highly divergent influenza subtypes. *PloS one* 6: e28001

650 Bodewes R *et al* (2013) In vitro assessment of the immunological significance of a human monoclonal antibody directed to the influenza a virus nucleoprotein. *Clinical and vaccine immunology : CVI* 20: 1333-1337

651 Shoji Y *et al* (2011) An influenza N1 neuraminidase-specific monoclonal antibody with broad neuraminidase inhibition activity against H5N1 HPAI viruses. *Human vaccines* 7 Suppl: 199-204

652 Grandea AG, 3rd *et al* (2010) Human antibodies reveal a protective epitope that is highly conserved among human and nonhuman influenza A viruses. *Proceedings of the National Academy of Sciences of the United States of America* 107: 12658-12663

653 HHS funds 2 experimental flu treatments. CIDRAP. http://www.cidrap.umn.edu/news-perspective/2015/09/hhs-funds-2-experimental-flu-treatments. Last Update September 29, 2015. Accessed November 8, 2015.

654 Visterra Pipeline. http://www.visterrainc.com/pipeline/pipeline.html. Last Update Accessed November 8, 2015.

655 HHS funds 2 experimental flu treatments. CIDRAP. http://www.cidrap.umn.edu/news-perspective/2015/09/hhs-funds-2-experimental-flu-treatments. Last Update September 29, 2015. Accessed November 8, 2015.

656 Pica N *et al* (2011) The DBA.2 mouse is susceptible to disease following infection with a broad, but limited, range of influenza A and B viruses. *Journal of virology* 85: 12825-12829

657 Kim JI *et al* (2013) DBA/2 mouse as an animal model for anti-influenza drug efficacy evaluation. *Journal of microbiology (Seoul, Korea)* 51: 866-871

658 van der Vries E *et al* (2013) Prolonged influenza virus shedding and emergence of antiviral resistance in immunocompromised patients and ferrets. *PLoS pathogens* 9: e1003343

659 Belser JA *et al* (2011) The ferret as a model organism to study influenza A virus infection. *Disease models & mechanisms* 4: 575-579

virulence, which limits their utility for the development of MCMs that can protect against severe disease. Moreover, the high genetic diversity among influenza viruses complicates the comparison of results from the use of two genetically diverse wild type strains that exhibit varying levels of pathogenicity.

9.7.4.6 Benefits and Limitations of Alt-GoF to Decision-Making in Public Health Policy

Evaluation of the virulence of circulating animal influenza viruses detected through surveillance informs assessment of their pandemic risk, which informs prioritization of investments in pre-pandemic preparedness initiatives, such as pre-pandemic vaccine development. The contribution of alt-GoF approaches to decision-making process in public health policy is discussed in detail in Section 9.6.4.3.

9.7.5 Comparison and Analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

In this section, the potential benefits of GoF research that enhances virulence *relative* to alt-GoF approaches are discussed, in each benefit category listed in the NSABB Framework.

9.7.5.1 Scientific Knowledge Benefits GoF Approaches Relative to Alt-GoF Approaches

9.7.5.1.1 Scientific Knowledge Gap 1: What Are the Viral Genetic and Phenotypic Traits That Underlie Pathogenicity in Mammals? What Are the Host Factors That Contribute to Enhanced Pathogenicity, as Well as Infection-Associated Morbidity and Mortality?

The underlying genetic and phenotypic features that result in infectivity, pathogenicity, and associated morbidity and mortality during influenza virus infection are poorly understood, in part because of the complex interplay between virus and host factors during pathogenesis. Because GoF and alt-GoF approaches have distinct benefits and limitations for the study of viral factors versus host factors that contribute to pathogenicity, their relative value for identifying and characterizing virus factors versus host factors is evaluated separately.

Identification and characterization of viral factors that contribute to pathogenicity

GoF approaches represent the most efficient and effective strategies for identifying novel viral genetic traits that contribute to the pathogenicity of any virus strain. In addition, targeted genetic modification of viruses to introduce traits associated with pathogenicity is uniquely capable of demonstrating that particular viral genetic traits are *necessary* and *sufficient* to enhance virulence across multiple virus contexts. However, results gleaned from cell culture and animal model studies may not translate to humans. Notably, the use of attenuated strains for these studies is hindered by the fact attenuation may alter disease pathogenesis, thus results may not be recapitulated in the genetic context of the wild type virus. In addition, attenuated strains cannot be used when the mechanism of attenuation alters the viral factor or underlying phenotype studied. However, the introduction of genetic traits associated with virulence to lab-adapted strains provides a controlled system for the dissection of the functions of individual genetic or phenotypic traits that contribute to virulence, and the fact that lab-adapted strains are attenuated permits investigation of a large spectrum of virulence. Finally, although the newly developed microRNA-based molecular biocontainment strategy is considered promising by the influenza research community, the fact that existing strategies restrict viral replication in humans and mice significantly limits the current utility of this strategy for pathogenicity studies, which often involve mice or human cell lines.

Although comparative sequence analysis of surveillance data has the potential to uncover viral genetic traits that are associated with virulence in humans, the utility of this approach is significantly

compromised by shortcomings in the quality and availability of surveillance data. Additionally, this approach is practically limited to the investigation of known viral genetic traits due to the high genetic diversity among influenza viruses. For the same reason, characterization of wild type isolates is limited to the study of previously known traits, unless genetically similar strains are available. In contrast, comparative analysis of isolates within patients enables the identification of novel adaptive traits that are associated with enhanced virulence over the course of infection. However this approach is often biased to severe and late stage infection, which may not be representative. LoF approaches also have limited utility for broad and unbiased identification of novel genetic and phenotypic traits due to their inefficiency, including the fact that LoF approaches may uncover traits that indirectly contribute to pathogenicity. Notably, targeted LoF enables the identification of genetic and phenotypic traits that are *necessary* for enhanced virulence, which provides valuable information to complement and strengthen results gleaned from targeted GoF studies.

While *in vitro,* virus free approaches and use of replication incompetent viruses enable the identification of novel genetic and phenotypic traits that are necessary and sufficient to alter phenotypes underlying pathogenicity, the importance of those genetic traits in the context of the complex host environment is difficult to extrapolate. Moreover, the *in vitro*, virus free and cell culture methods do not provide any information on mechanisms underlying the morbidity and mortality associated with influenza infection.

Finally, host-focused approaches provide indirect insight into the function of virus proteins and thus are of limited utility for understanding how viral factors contribute to pathogenicity, relative to GoF approaches.

Identification and characterization of host factors that contribute to pathogenicity

Both GoF and alt-GoF approaches can provide insight into host factors that enhance pathogenicity, including deleterious immune responses that contribute to the morbidity and mortality caused by influenza infection. GoF approaches can be used to identify host factors that are *associated* with enhanced virulence and morbidity and mortality. In particular, targeted genetic modification to introduce traits that are expected to enhance virulence provides a controlled system that can be used to tease apart the interplay between virus and host factors contributing to pathogenesis, i.e., by demonstrating how changes to a particular virus factor alter host immune responses and enhance infection-associated-pathology. The utility of using risk-mediation reassortants in lieu of wild type viruses is significantly limited for the study of host factors that contribute to pathogenicity due to differences in underlying pathogenesis mechanisms. The main drawback of GoF approaches, with respect to the study of *host* factors that contribute to pathogenicity, is that they cannot establish a causal link between a host factor and enhanced pathogenicity and/or more severe disease pathology. Additionally, results from representative animal models may not translate to humans.

The use of targeted knockout animals or pharmacological inhibition of the host factor during infection, an alt-GoF approach, is uniquely capable of confirming that a host factor contributes to virulence and pathogenicity. However, because the host response is dynamic and complex, inhibition of a host factor is likely to have a multi-faceted effect on immune responses during infection, making the identification of host traits that contribute to virulence difficult to resolve. Targeted genetic modification of viruses to introduce traits expected to attenuate virulence (LoF) can also be used to identify host factors/responses that are associated with enhanced pathogenicity. Like its GoF counterpart (i.e., targeted genetic modification of viruses to introduce traits expected to enhance virulence), this approach provides a controlled system for studying interplay between virus and host factors contributing to pathogenesis, and the resulting information complements results from GoF studies. Immunological characterization of wild type isolates exhibiting varied levels of virulence can demonstrate an association between a particular host response and exacerbated disease pathology. However, this approach provides little mechanistic

insight into the role of particular virus-host interactions if viral isolates display high genetic diversity. Several other alt-GoF approaches provide correlative data about the course of disease and the immune responses that are associated with severe outcomes observed in humans, including comparative analysis of genetic surveillance data, analysis of patient isolates, and analysis of autopsy data. This information is highly valuable for connecting results observed in animal model systems to nature (e.g., whether neurotropism observed during infections of ferrets with H5N1 viruses is representative of human infections). However, these approaches provide limited mechanistic insight and are impaired by limitations in the quality and availability of genetic surveillance data.

9.7.5.1.2 Scientific Knowledge Gap 2: Discover Whether Fitness Defects Associated with the Acquisition of Antiviral Resistance Can Be Overcome, and the Mechanisms Underlying Recovery of Fitness

GoF approaches are uniquely capable of proactively discovering compensatory mutations that rescue the fitness of any antiviral-resistant strain with impaired growth, as well as establishing a causal link between compensatory mutations and enhanced fitness. Computational modeling can be used to generate hypotheses about mutations that may rescue growth, but all predictions must be experimentally confirmed using GoF approaches. Comparative sequence analysis of antiviral-resistant strains with varied levels of fitness has significant limitations relative to other approaches.

9.7.5.1.3 Scientific Knowledge Benefit 3: Generation of Animal Models for the Study of Flu-Associated Morbidity/Mortality

Model systems that can be efficiently infected by influenza viruses and exhibit the spectrum of disease observed during human infections are essential for the study of influenza-associated morbidity/mortality. Although the ability to infect wild type hosts with wild type viruses would be ideal for translation of the results of pathogenesis studies to human populations, mice are naturally resistant to infection with many influenza viruses and/or wild type viruses may display a limited spectrum of disease in mice and ferrets. In these cases, because pathogenicity and disease outcome is dependent on the interplay between virus and host, both GoF and alt-GoF approaches enable the development of model systems that expand the dynamic range of pathogenesis that is observed when using wild type viruses and wild type hosts. GoF approaches achieve this goal by enhancing the virulence of the virus through serial passaging, while alt-GoF approaches enhance host susceptibility to disease through targeted genetic modification or the use of immunosuppressants. Both strategies generate animal models exhibiting a wider spectrum of disease than observed in nature, which can be used to tease apart the relationship between host immune responses and infection-associated morbidity and mortality. However, both GoF and alt-GoF approaches have limitations. Serial passaging (GoF) may change the phenotypic properties of the virus in ways that alter its biology, which could lead to misrepresentative findings. Modification of the host (alt-GoF) may alter host immune responses that are involved in the response to infection, complicating translation of findings to disease in healthy hosts. The genetic modification approach is limited to mice, although the use of immunosuppressants represents a promising approach for ferrets, which are better representative of human disease. Given these caveats, the use of model systems derived from GoF and alt-GoF approaches strengthens the validity of any findings.

9.7.5.2 Surveillance Benefits of GoF Approaches Relative to Alt-GoF Approaches

The strategies for monitoring the virulence of circulating animal influenza viruses detected through surveillance are similar to those for monitoring mammalian adaptation and transmissibility, and GoF and alt-GoF approaches benefit surveillance through similar mechanisms. Thus, the relative benefits are discussed collectively in Section 9.6.5.2.

9.7.5.3 Vaccine Development Benefits of GoF Relative to Alt-GoF Approaches

9.7.5.3.1 Vaccine Development Benefit 1: Development of New Influenza Vaccine Candidates

A variety of vaccine platforms are being explored for the development of vaccines targeting avian influenza viruses with pandemic potential. LAIVs have several characteristics that are desirable for pandemic vaccines, but a major concern associated with their use is that the LAIV may recover virulence upon growth in people. GoF approaches are uniquely capable of demonstrating whether LAIV strains recover virulence upon growth *in vivo*, a critical aspect of vaccine safety testing prior to the conduct of clinical trials. Other types of vaccines in development have strengths and weaknesses relative to LAIVs. The type or types of vaccines that will ultimately prove to be most effective for avian influenza viruses is not yet clear based on vaccinology research conducted to date. Given the need for effective pandemic influenza vaccines, pursuing all promising strategies for vaccine development in tandem, including LAIVs, will ensure that an effective vaccine is achieved in the shortest possible period of time.

9.7.5.3.2 Vaccine Development Benefit 2: Targeted Mutagenesis to Remove Virulence Markers from Vaccine Viruses

GoF approaches represent the most efficient and effective strategies for discovering novel genetic traits that contribute to the virulence of influenza viruses. However, GoF approaches cannot be used to identify or confirm genetic changes that are sufficient to *attenuate* the virulence of wild type strains, which is the goal of vaccine virus development. LoF approaches, namely targeted mutagenesis, are uniquely capable of identifying genetic changes (mutations or deletions) attenuate virulence across multiple virus strains. Taken together, these approaches may enable the identification of novel virulence traits that can be mutated to attenuate virulence, which can be applied to the production of AI vaccine viruses to further improve the safety of the vaccine production process.

9.7.5.4 Therapeutic Development Benefits of GoF Approaches Relative to Alt-GoF Approaches

GoF approaches represent the most efficient and effective strategy for discovering novel viral virulence factors that may be good therapeutic targets, but follow-up alt-GoF approaches are needed to confirm that inhibiting the function of a particular viral factor is sufficient to attenuate or block viral replication and/or reduce infection-associated pathology. Alt-GoF approaches are best-suited for discovering novel host factors that contribute to virulence and immunopathology. However, GoF approaches can be used to gain further mechanistic insight into the function of the host protein during infection, which strengthens the evidence base for developing new therapeutics targeting that host factor. Two completely different approaches for generating new therapeutic candidates are screening libraries of small molecule compounds for their ability to inhibit viral replication *in vitro* and isolating monoclonal antibodies that neutralize essential virus activities by directly binding to virus proteins, both of which have generated promising therapeutic candidates that have entered clinical trials. Given that influenza viruses readily acquire mutations that confer resistance to therapeutics and that different types of therapeutics may be most effective against various influenza sub-types, a wide repertoire of therapeutics is needed to best protect the public against the range of influenza threats that exist in nature. Pursuing all promising pathways for therapeutic development in tandem, including GoF approaches, is the best strategy to achieve this goal.

9.7.5.5 Benefits of GoF Approaches Relative to Alt-GoF Approaches for the Development of Both Vaccines and Therapeutics

Model systems that can be efficiently infected by influenza viruses and exhibit the spectrum of disease observed during human infections are essential for testing the safety and efficacy of new vaccines and

therapeutics. Although the ability to infect wild type hosts with wild type viruses would be ideal for translation of the results of MCM development studies to human populations, mice are naturally resistant to infection with many influenza viruses and/or wild type viruses may display a limited spectrum of disease in mice and ferrets. In these cases, because pathogenicity and disease outcome is dependent on the interplay between virus and host, both GoF and alt-GoF approaches enable the development of model systems that expand the dynamic range of pathogenesis that is observed when using wild type viruses and wild type hosts. GoF approaches achieve this goal by enhancing the virulence of the virus through serial passaging, while alt-GoF approaches enhance host susceptibility to disease through targeted genetic modification or the use of immunosuppressants. Both approaches provide a controlled system for comparing the effectiveness of MCM candidates to protect against more severe disease, and both have limitations. Serial passaging (GoF) may change the phenotypic properties of the virus in ways that alter its susceptibility to the MCM in development, which would lead to misrepresentative findings. Modification of the host (alt-GoF) may alter host immune responses that are involved in the mechanism of action of the vaccine or therapeutic, complicating translation of findings to disease in healthy hosts. The genetic modification approach is limited to mice, although the use of immunosuppressants represents a promising approach for ferrets, which are better representative of human disease. Given these caveats, the use of model systems derived from GoF and alt-GoF approaches strengthens the validity of any findings.

9.7.5.6 Benefits to Decision-Making in Public Health Policy

The relative contribution of GoF and alt-GoF approaches to benefit the decision-making process in public health policy is discussed in detail in Section 9.6.5.3, as evaluation of the transmissibility of animal influenza viruses similarly informs pandemic risk assessments and downstream decision-making.

9.8 Influenza Viruses: Benefits of GoF Research That Leads to Evasion of Existing Natural or Induced Adaptive Immunity

9.8.1 Summary

This section describes the benefits of GoF research that is reasonably anticipated to lead to evasion of existing natural or induced adaptive immunity. Such GoF studies were found to generate scientific knowledge, to inform surveillance of circulating seasonal influenza viruses, which has downstream benefits to the production of seasonal influenza vaccines, and to benefit the development of new types of influenza vaccines. Alt-GoF approaches that may generate similar benefits were also identified and analyzed. At present, GoF studies resulting in evasion of existing natural or induced adaptive immunity have unique benefits to scientific knowledge and surveillance, though full realization of GoF benefits to surveillance requires scientific advancements and expansion of global public health surveillance networks. Chapter 9.8 provides an overview of these benefits, including basic background and Supporting Information; a fully referenced and more thorough discussion of these benefits can be found in Appendix IV Section 15.5.

9.8.1.1 Benefits of GoF Research to Scientific Knowledge

- GoF approaches:
  - Are uniquely capable of providing in-depth information about the evolutionary mechanisms driving antigenic drift as well as prospective information about currently circulating influenza viruses. However, laboratory results may not translate to the evolution of flu viruses in human populations.

- Are the most reliable and efficient method for discovering amino acid substitutions that confer antigenic change to circulating viruses and are uniquely capable of demonstrating that particular amino acid substitutions are necessary and sufficient to alter antigenicity. However, these insights can be gleaned using attenuated 6:2 reassortant strains in lieu of wild type viruses.

- Are the only method for mapping the antigenic sites of the HA protein in the context of the full virus but are relatively low-throughput.

- Alt-GoF approaches:
  - Are uniquely capable of providing information about the antigenic evolution of influenza viruses in nature but are constrained to studying the evolution of historic viruses in limited depth.

  - Allow for high-throughput mapping of antigenic sites using virus-free approaches, but results may not be recapitulated in the context of the full virus.

9.8.1.2 Benefits of GoF Research to Surveillance

- GoF approaches:
  - Are uniquely capable of strengthening the predictive value of molecular markers for antigenic change, which can be used to infer phenotype from sequence. Use of molecular markers in lieu of or to corroborate phenotypic testing results could improve the quality, timeliness, and quantity of antigenic information about seasonal flu viruses detected through surveillance. However, the success of this approach is subject to significant advancements in the state of knowledge about the molecular basis of antigenic differences.

  - Are critical for improving computational models for predicting antigenic phenotype based on sequence. Use of computational models in lieu of or to corroborate phenotypic testing results could improve the quality, timeliness, and quantity of antigenic information about seasonal flu viruses detected through surveillance. However, the success of this approach is subject to significant advancements in the accuracy of existing models.

  - Full realization of these GoF benefits will require expansion of sequencing capabilities diagnostic labs involved in global influenza surveillance.

- Alt-GoF approaches:
  - Have significant limitations for strengthening the predictive value of molecular markers for antigenic changes.

  - Are also critical for improving computational models for predicting antigenic phenotype based on sequence, but through the generation of different types of data that complement data generated through GoF approaches.

  - Phenotypic assays for antigenic characterization are uniquely capable of providing direct information about antigenicity, but results may be delayed relative to the publication of viral sequences.

9.8.1.3 Benefits of GoF Research to Vaccine Development

- GoF approaches:

- GoF approaches that improve sequence-based predictions of antigenicity have potential to increase the robustness, quantity, and timeliness of antigenic characterization data upon which strain selection decisions are based. However, full realization of this benefit depends on the expansion of sequencing capabilities at National Influenza Centres.
- GoF approaches have potential to improve the ability to predict antigenic drift, through experimental and/or computational methods, which would allow the production of "antigenically advanced" vaccines that match circulating strains at their time of deployment. However, the success of this approach is subject to significant advancements in the state of knowledge about the evolutionary mechanisms driving antigenic drift.
- Are uniquely capable of defining the antigenic landscape of the HA protein (the spectrum of antigenic configurations that HA can assume and which regions of HA can mutate while preserving virus viability). These data may inform the development of broad-spectrum or universal flu vaccines.

- Alt-GoF approaches:
  - Efforts to improve antigenic characterization assays, in order to improve the quality of antigenic characterization data upon which strain selection decisions are based, are ongoing but have had limited success to date.
  - Strengthening global influenza surveillance networks will improve the quantity, timeliness, and representativeness of data upon which strain selection decisions are based, but these efforts face considerable funding and political barriers.
  - Alternative strategies for the development of broad-spectrum or universal flu vaccines are being pursued and have also shown promise.

9.8.2 Overview of GoF Research Landscape: Evasion of Existing Natural or Induced Adaptive Immunity

9.8.2.1 Serial Passaging of Viruses in the Presence of Cognate Antibodies

Serial passaging of viruses in the presence of cognate antibodies may lead to the acquisition of mutations that allow the virus to escape neutralization by the antibody. This experiment can be performed in cell culture using monoclonal antibodies, convalescent sera from infected individuals, post-infection ferret sera, or in animals that have been vaccinated or previously exposed to influenza viruses. Sequencing of emergent antibody escape viruses identifies amino acid substitutions that are sufficient to confer antigenic change, which provides a foundation for follow-up studies investigating the molecular basis of antigenic differences between strains. Additionally, sequencing viral isolates at multiple stages of the selection process and determining the effect of amino acid substitutions on viral fitness and other virus phenotypes provides insight into the evolutionary mechanisms driving antigenic drift. Finally, when performed *in vitro* using monoclonal antibodies, the location of escape mutations reveals potential antibody epitope sites.

9.8.2.2 Forward Genetic Screen to Identify Mutations That Alter Antigenicity

Forward genetic screens involve random mutagenesis of the HA protein followed by characterization of the antigenicity of mutants using the hemagglutination inhibition (HAI) assay or other assays, in order to identify amino acid substitutions that do and do not lead to antigenic change. Follow-up studies may

determine the consequences of antigenicity-altering mutations on other virus phenotypes, such as viral fitness and pathogenicity. As for serial passaging experiments, the identification of amino acid substitutions that confer antigenic change provides a foundation for studies investigating the molecular basis of antigenic differences. In addition, comprehensive forward genetic screens can be used to define the 'antigenic landscape' of the HA protein – that is, which substitutions the HA protein will tolerate and which of those substitutions cause antigenic drift.

9.8.2.3 Targeted Modification of Viruses to Introduce Mutations That Are Expected to Alter Antigenicity

A final GoF approach that may lead to viruses that evade existing adaptive immunity involves targeted genetic modification to introduce mutations that are expected to alter antigenicity, followed by antigenic characterization of the mutant virus using the HAI assay or other assays. Of note, mutations may be identified through GoF approaches, such as serial passaging of viruses in the presence of cognate antibodies, or alt-GoF approaches, such as comparative analysis of historical sequences. This approach demonstrates that a particular mutation or set of mutations is necessary and sufficient to alter antigenicity, which provides a foundation for follow-up studies investigating the molecular basis of antigenic differences between strains.
Notably, the level of pre-existing immunity to a given wild type influenza virus in the human population varies depending on when the strain circulated in human populations and other factors. For example, only those people born prior to or shortly after the 1968 H3N2 influenza pandemic may possess pre-existing immunity to the 1968 H3N2 virus today, acquired through exposure to the 1968 strain or antigenically similar descendants by natural infection or vaccination. In contrast, a large fraction of the population is expected to have pre-existing immunity to recently or currently circulating seasonal influenza viruses or to seasonal influenza viruses that have recently served as the basis for vaccine strains. Consequently, the degree to which laboratory-generated strains that evade pre-existing immunity, created using any one of the GoF approaches described above, pose an increased risk to human health at the population level is strain-specific (i.e., depends on the history of that virus strain and the level of existing immunity in the human population).

With this caveat in mind, the scope of the benefit assessment for this GoF phenotype includes seasonal and pandemic influenza viruses. (Pandemic influenza viruses include the 1918 H1N1 pandemic virus, the 1957 H2N2 pandemic virus, and the 1968 H3N2 virus, but not the 2009 H1N1 pandemic (H1N1pdm) virus, which is now circulating seasonally.) Of note, although only a small (elderly) fraction of the population has pre-existing immunity to the 1918 H1N1 pandemic virus through natural exposure to the 1918 strain or its early descendants, vaccination against the 2009 H1N1pdm virus has been shown to afford cross-protection against the 1918 H1N1 virus. Specifically, vaccination of mice or ferrets using the monovalent or trivalent form of the inactivated 2009 H1N1pdm vaccine reduced morbidity and mortality associated with subsequent infection with the 1918 H1N1 pandemic virus.[660,661,662] (For a more detailed description of these data, see the online supplemental material.) These data, coupled with the fact that most neutralizing antibodies elicited by infection with H1N1pdm have been found to be broadly neutralizing (against strains as divergent as H5N1),[663] strongly suggest that natural infection with the 2009 H1N1pdm virus would also cross-protect against infection with the 1918 H1N1 virus.[664] However,

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660 Easterbrook JD *et al* (2011) Immunization with 1976 swine H1N1- or 2009 pandemic H1N1-inactivated vaccines protects mice from a lethal 1918 influenza infection. *Influenza Other Respir Viruses* 5: 198-205
661 Medina RA *et al* (2010) Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus. *Nat Commun* 1: 28
662 Pearce MB *et al* (2012) Seasonal trivalent inactivated influenza vaccine protects against 1918 Spanish influenza virus infection in ferrets. *Journal of virology* 86: 7118-7125
663 Wrammert J *et al* (2011) Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med* 208: 181-193
664 Personal communications from influenza researchers (January 2016).

this phenomenon has not yet been formally investigated. Taken together, this body of research suggests that the US and global populations may have significant pre-existing immunity to the 1918 H1N1 virus, though how and whether such immunity would mitigate the consequences of an outbreak caused by the 1918 virus is uncertain. For this reason, antigenic escape studies utilizing the 1918 H1N1 virus and its early descendants were included in the analysis of the benefits of GoF research that leads to evasion of existing natural or induced immunity. To the authors' knowledge, such studies have not been performed utilizing the reconstructed 1918 H1N1 virus. However, several antigenic escape studies involving a classical swine H1N1 isolate from 1930 (A/Swine/Iowa/15/30), the HA sequence of which more closely resembles the 1918 HA sequence than the sequence of any other existing isolate,[665] were identified. These studies are included in the landscape tables for the "Evasion of Existing Natural or Induced Immunity" section (Supplemental Information) and their benefits are evaluated here. Of note, this 1930 strain is not known to infect humans, although more recent classical swine influenza viruses can infect people.

In contrast, because human populations do not have widespread immunity to animal influenza viruses (i.e., avian viruses[666] and swine viruses[667]), no approaches involving these viruses meet this phenotypic criterion. Therefore, this section does not include studies that investigate the mechanisms underlying antigenic drift of avian strains in response to selection pressure from vaccination or the chicken immune system, nor any other studies focused on animal influenza strains. Note that because these studies may lead to the acquisition of mutations in the influenza HA protein, which is a critical determinant of mammalian adaptation, transmissibility, and virulence, these studies may result in the generation of viruses with altered virulence, infectivity, and transmissibility from a "human" perspective. However, whether and what phenotypic changes are likely to arise cannot be anticipated with certainty.

Finally, GoF approaches may also lead to the generation of influenza viruses that are capable of evading recognition by the host innate immune system. Because virus interactions with innate immune factors are critical determinants of virulence, these approaches are evaluated in the "enhanced morbidity and mortality in appropriate animal models" section (9.7).

9.8.3 Identification of the Potential Benefits and Limitations of GoF Approaches

9.8.3.1 Benefits and Limitations of GoF Approaches to Scientific Knowledge

In this section, the ability of GoF methods to address three unanswered questions in this field are evaluated:

- How do influenza viruses evolve antigenically in response to immune pressure? That is, what are the evolutionary mechanisms driving antigenic drift, including the role of different selection pressures (e.g., vaccination) and the interplay between antigenic escape and other virus phenotypes, such as fitness?
- What is the molecular basis of antigenic drift? That is, what amino acid substitutions in the HA protein lead to antigenic change, and what is the biophysical basis of that effect?
- What are the antigenic sites on the HA protein that are targeted by neutralizing antibodies?

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665. Yu X *et al* (2008) Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 455: 532-536
666. Jernigan DB, Cox NJ (2015) H7N9: Preparing for the Unexpected in Influenza. *Annual Review of Medicine* 66: 361-371
667. Skowronski DM *et al* (2012) Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2v). *J Infect Dis* 206: 1852-1861

Influenza viruses circulating in nature acquire mutations in response to immune pressure from human populations that allow the viruses to escape recognition by the adaptive immune system, a process termed "antigenic drift".[668] As a result, the strain composition of the seasonal influenza vaccine must be updated annually to ensure that the vaccine strains antigenically "match" circulating strains. Research in this area is focused on the influenza HA protein, which is the immunodominant influenza protein and represents the primary component of current influenza vaccines. The mechanisms underlying antigenic drift of the HA protein and the relationship between genotype and antigenic phenotype are not well understood. One of the knowledge gaps that contributes to this uncertainty is an incomplete understanding of the antigenic sites on the HA protein that are targeted by neutralizing antibodies, as these sites are presumably hotspots for antigenic evolution.[669] Mapping antigenic sites is also important for understanding the molecular basis of neutralizing antibody activity, as well as gaining insight into the mechanisms underlying the cross-protection afforded by broadly neutralizing antibodies (e.g., neutralizing antibodies produced in response to the 2009 H1N1 pandemic virus afford some level of protection against infection with the 1918 H1N1 pandemic virus, which has a related HA sequence, and vice versa).[670,671,672,673,674]

9.8.3.1.1 Scientific Knowledge Gap 1 – How Do Influenza Viruses Evolve Antigenically in Response to Immune Pressure?

GoF approaches that involve serial passaging of viruses in the presence of cognate antibodies provide insight into the evolutionary mechanisms driving antigenic drift in response to immune pressure. Both *in vivo* and *in vitro* approaches have unique strengths. Namely, subjecting viruses to selection from the full complement of the animal immune system better mimics the selective pressure viruses experience in humans, while *in vitro* approaches can be conducted using convalescent sera (or isolated antibodies) from people, which may be more relevant to humans than selective pressures in animals. In addition, the *in vivo* approach represents a controlled system for studying the role of selective pressures from prior exposure to influenza viruses through natural infection and/or vaccination in shaping antigenic evolution. In both cases, results from laboratory studies may not translate to the evolution of viruses in human populations in nature and may not be conserved in other virus contexts. Importantly, follow-up studies can determine the effect of antigenic drift on other virus phenotypes, such as fitness, which provides insight into how likely mutations are to persist in a host or in a population once they have arisen.

9.8.3.1.2 Scientific Knowledge Gap 2 – What Is the Molecular Basis of Antigenic Drift?

Several GoF approaches can be used to discover mutations that lead to antigenic drift, which provides a foundation for follow-up studies investigating the biophysical basis of antigenic change. First, serial passaging of viruses in cells in the presence of cognate sera or monoclonal antibodies, or in animals that have been vaccinated or previously exposed to influenza viruses, leads to the emergence of antigenic escape mutants. Sequencing the HA gene of emergent escape viruses reveals mutations that are sufficient to alter virus antigenicity. This approach is highly efficient and can be applied to any virus, including currently circulating strains. Notably, *in vitro* and *in vivo* selection approaches equally enable the

668 Webster RG *et al* (1982) Molecular mechanisms of variation in influenza viruses. *Nature* 296: 115-121
669 O'Donnell CD *et al* (2012) Antibody pressure by a human monoclonal antibody targeting the 2009 pandemic H1N1 virus hemagglutinin drives the emergence of a virus with increased virulence in mice. *MBio* 3
670 Medina RA *et al* (2010) Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus. *Nat Commun* 1: 28
671 Easterbrook JD *et al* (2011) Immunization with 1976 swine H1N1- or 2009 pandemic H1N1-inactivated vaccines protects mice from a lethal 1918 influenza infection. *Influenza Other Respir Viruses* 5: 198-205
672 Pearce MB *et al* (2012) Seasonal trivalent inactivated influenza vaccine protects against 1918 Spanish influenza virus infection in ferrets. *Journal of virology* 86: 7118-7125
673 Manicassamy B *et al* (2010) Protection of mice against lethal challenge with 2009 H1N1 influenza A virus by 1918-like and classical swine H1N1 based vaccines. *PLoS Pathog* 6: e1000745
674 Wei CJ *et al* (2010) Cross-neutralization of 1918 and 2009 influenza viruses: role of glycans in viral evolution and vaccine design. *Sci Transl Med* 2: 24ra21

identification of mutations associated with antigenic drift, though the *in vitro* approach is faster and cheaper. Importantly, as multiple mutations may arise during passaging, follow-up studies may be needed to determine which mutation(s) are responsible for the antigenic escape phenotype.

Forward genetic screens, which involve mutagenesis of the HA protein and subsequent characterization of the antigenicity of mutant viruses, represent another GoF approach for identifying mutations that confer antigenic change. Though screening for escape mutants is more labor-intensive than selection methods based on serial passaging, the screening approach is uniquely capable of identifying mutations that do *not* lead to antigenic change, which critically informs efforts to develop models for the sequence-based prediction of antigenicity. Importantly, because of the influence of genetic context on antigenicity, antigenic escape mutations identified through either serial passaging or forward genetic screens may not generalize to other virus strains within the same or different HA subtype.

Finally, targeted genetic modification of viruses to introduce mutations associated with antigenic change, followed by antigenic characterization of mutant viruses, is used to demonstrate that mutations are *necessary* and *sufficient* to alter antigenicity. Subsequently, to determine whether the phenotypic consequences of mutations are functionally generalizable across multiple virus strains, targeted mutagenesis can be used to introduce mutations into new virus strains, followed by antigenic characterization. Together, these results provide a strong foundation for follow-up structural studies to determine the biophysical basis of antigenic differences and critically inform the development of models for the prediction of antigenic phenotype from genotype.

9.8.3.1.3 Scientific Knowledge Gap 3 – What Are the Antigenic Sites on the Ha Protein That Are Targeted by Neutralizing Antibodies?

Serial passaging of viruses in cells in the presence of monoclonal antibodies (mAbs) to select for antibody escape mutants is a classic method for identifying putative antibody binding sites. Specifically, the amino acid positions where mutations arise represent potential antigenic sites, although interpretation of this data is complicated by the fact that mutations outside antibody binding sites can impact HA-antibody interactions through long-range effects. In the event that multiple mutations arise within the HA protein, targeted mutagenesis to introduce individual mutations into the parental strain may be used to confirm which mutations are necessary and sufficient to confer escape. This approach is simple, rapid, and allows for precise mapping of antigenic sites. However, each passaging experiment focuses on the identification of a single antigenic site (i.e., recognized by a particular mAb), such that multiple rounds of passaging with distinct antibodies are required to map multiple antigenic regions.

9.8.3.2 Benefits and Limitations of GoF Approaches to Surveillance

GoF approaches that lead to the identification of mutations that alter antigenicity have potential to aid antigenic surveillance of human seasonal influenza viruses by facilitating prediction of antigenic phenotype from genotype, in lieu of isolating and experimentally evaluating the antigenicity of viruses. Specifically, GoF data can strengthen the predictive value of molecular markers for antigenic change and can improve models for predicting antigenic phenotype from genotype. Either application has the potential to aid the bi-annual selection of strains for the seasonal influenza vaccine, as described in the "informing policy decisions" section below.

9.8.3.2.1 Introduction to Influenza Virus Surveillance: Current Practices and Limitations

The WHO Global Influenza Surveillance and Response System (GISRS) conducts surveillance of seasonal influenza viruses year-round. The major goal of seasonal flu surveillance is to monitor the antigenic evolution of viruses – that is, to detect when new antigenic variants emerge in human

populations and to determine their prevalence and geographic distribution.[675,676] A global network of National Influenza Centres (NICs) collect clinical specimens in their countries and ship viral isolates to one of six WHO Collaborating Centres (WHOCCs) for detailed antigenic characterization.[677,678] These data critically inform WHO-coordinated decisions about which strains to recommend including in the seasonal flu vaccine, which are developed during bi-annual Vaccine Composition Meetings (VCMs).[679,680] If surveillance data indicate that a new antigenic variant has emerged and spread geographically, the WHO strain selection committee will recommend updating that component of the vaccine.

Antigenic characterization primarily relies on the hemagglutination inhibition (HAI) assay developed in the 1940s.[681] Though simple and inexpensive, HAI assays have several significant drawbacks that compromise their utility and reliability for antigenic characterization.[682,683] GoF approaches have potential to address this shortcoming by improving two methods for predicting antigenic phenotype based on sequence, thereby improving antigenic surveillance of seasonal influenza viruses. First, HA sequences can be inspected for the presence or absence of molecular markers for antigenic drift that were identified through GoF approaches. Second, that same GoF-derived data can be used to improve existing models for predicting antigenicity based on genotype. In either case, that information could supplement phenotypic characterization data, to strengthen the certainty of conclusions about antigenic relationships between strains, or could be used in lieu of phenotypic characterization data.

9.8.3.2.2 Analysis of GoF Approaches That Support the Use of Molecular Markers to Evaluate the Antigenicity of Seasonal Influenza Viruses

During the current strain selection process, HA sequences are inspected for the presence of amino acid substitutions that are known to be associated with altered antigenicity. This information can be used to corroborate antigenic characterization data from the HAI assay or can help to resolve antigenicity questions when HAI assay results are difficult to interpret. While this information informs the decision-making process, the utility of these markers is limited by significant uncertainties in the state of this science. First, the ability to reliably predict whether a particular amino acid substitution will confer antigenic change in a new genetic context is poor. Second, because other, as-yet-undiscovered amino acid changes may alter antigenicity, the absence of known markers is not yet meaningful (i.e., does not indicate that the antigenicity of the strain is unchanged).

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675 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

676 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

677 (2015p) Interview with Centers for Disease Control and Prevention representative

678 WHO. Global Influenza Surveillance and Response System (GISRS). http://www.who.int/influenza/gisrs_laboratory/en/. Last Update Accessed December 7, 2015.

679 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

680 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

681 Hirst GK (1942) THE QUANTITATIVE DETERMINATION OF INFLUENZA VIRUS AND ANTIBODIES BY MEANS OF RED CELL AGGLUTINATION. *J Exp Med* 75: 49-64

682 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

683 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

GoF approaches are critical for addressing both aspects of scientific uncertainty described above to strengthen the utility of molecular marker data for antigenic change. To strengthen the predictive value of molecular markers for antigenic change, several types of experiments are needed:

- Targeted mutagenesis to introduce known genetic markers for altered antigenicity into new genetic contexts (i.e., validate the antigenic consequences of the marker in a variety of strain contexts), which represents a GoF approach,
- Targeted mutagenesis to determine which amino acid substitutions at a particular site previously associated with antigenic change are sufficient to alter antigenicity, which represents a GoF approach, and
- Experiments that explore the antigenic plasticity of the HA protein, to discover new substitutions that confer antigenic change as well as substitutions that do not alter antigenicity.

To address the third experimental goal, two GoF approaches (serial passaging and forward genetic screens) are capable of uncovering novel mutations that confer antigenic change, and targeted mutagenesis can be used to confirm their causality (also GoF). Although these data will undoubtedly strengthen the predictive value of molecular markers for antigenic change, given the importance of genetic context on influenza biology, significant challenges face any effort to improve the predictive value of such markers to a level that is meaningful. Whether this goal is achievable will depend on whether the number of amino acid substitutions that HA can accept is limited or very large, which is as-yet-unknown. In addition, the fact that negative results are generally not published in the scientific literature also hinders advancements in this area, as knowing when markers are not conserved critically informs their utility.

9.8.3.2.3 Analysis of GoF Approaches That Improve Predictive Models

GoF data can also be used to improve the quality of computational models for predicting antigenic phenotype from genotype, which represents a different sequence-based approach for predicting antigenicity. Current models cannot accurately predict antigenic phenotype from genotype.[584]

GoF approaches have potential to improve these models in two ways: (1) by generating experimental data about novel antigenic changes that are necessary and sufficient to alter antigenicity, which can be incorporated into datasets used to train the models, and (2) by testing predictions of novel mutations that would affect antigenicity that these models make, the results from which will feed back to improve model accuracy. As existing models are primarily trained using historical data (i.e., the sequences and antigenic characterization data from historical isolates), the ability of GoF approaches to explore new antigenic space will complement existing data sources to enhance the predictive capability of these models for currently circulating isolates that are evolving antigenically in new ways. As above, the feasibility of developing models that can accurately predict antigenic phenotype from genotype will depend on the antigenic plasticity of the HA protein, which is currently unknown.

If the landscape of amino acid substitutions that can give rise to antigenic change is large, then molecular markers and computational models may never be robust enough to replace antigenic characterization data generated through laboratory assays. Nonetheless, given the shortcomings of phenotypic assays for characterizing antigenicity, the ability to corroborate laboratory results using sequence-based predictions

[584] (2015) Interviews with influenza researchers.

can significantly strengthen the quality of antigenic characterization data, particularly if clinical specimens are directly sequenced.

9.8.3.3 Benefits and Limitations of GoF Approaches to Vaccine Development

9.8.3.3.1 Vaccine Development Benefit 1: Improve Strain Selection Capabilities for Seasonal Influenza Vaccines

GoF approaches have potential to improve the strain selection process for seasonal influenza vaccines in several ways. First, a critical factor in strain selection is analysis of the antigenic characteristics of circulating influenza viruses, to determine whether new antigenic variants have emerged. As described in Section 9.8.3.2, GoF data can improve methods for predicting antigenic phenotype from genotype, which may provide several advantages over the use of traditional, laboratory-based antigenic characterization methods. In addition, GoF approaches have the potential to aid efforts to predict antigenic drift, either directly through the selection and analysis of drifted strains or by informing the development of models for predicting drift. As selected strains sometimes drift during the course of vaccine development, which leads to poor vaccine match, these efforts could improve the efficacy of vaccines by enabling deliberate production of "drifted" strains that match circulating strains at the time of vaccine deployment.

Introduction to strain selection for seasonal flu vaccines: current practice and limitations

Since the early 1970s, the WHO has provided formal recommendations for the strain composition of seasonal influenza vaccines based on year-round influenza surveillance conducted through the GISRS (described above).[685,686] Experts must predict which strains are likely to be dominant six to eight months in advance of the start of the target flu season, to provide sufficient time for manufacturing the vaccine.[687,688] Despite the complexity of the data considered and the challenge of predicting dominant strains many months in advance, this process generally works well– most years, the vaccine is well-matched to circulating strains.[689] However, occasionally a rare antigenic variant rises to prominence during the course of vaccine production, as happened during the recent 2014– 2015 flu season for the H3N2 strain, which results in poor vaccine match and reduced vaccine efficacy.[690,691]

Several shortcomings compromise the efficacy of the current strain selection process. First, the timeliness and representativeness of isolates forwarded to WHOCCs by NICs, which form the basis of strain selection recommendations, could be improved. In particular, due to significant lag times between sample collection and shipment (e.g., two to three months between 2010 and 2012 in the WHOCC London region), many isolates cannot be analyzed in time for consideration during VCM meetings. These

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685 Oshitani H (2010) Influenza surveillance and control in the Western Pacific Region. *Western Pacific surveillance and response journal : WPSAR* 1: 3-4

686 WHO. Process of Influenza Vaccine Virus Selection and Development. http://apps.who.int/gb/pip/pdf_files/Fluvaccvirusselection.pdf. Last Update November 19, 2007. Accessed November 22, 2015.

687 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

688 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

689 Legrand J *et al* (2006) Real-time monitoring of the influenza vaccine field effectiveness. Ibid. 24: 6605-6611

690 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. Ibid. 31: 3209-3221

691 Xie H *et al* (2015) H3N2 Mismatch of 2014-15 Northern Hemisphere Influenza Vaccines and Head-to-head Comparison between Human and Ferret Antisera derived Antigenic Maps. *Sci Rep* 5: 15279

shortcomings in existing surveillance networks reduce the quality and quantity of input data for strain selection decisions, which compromises the accuracy of the process. A second shortcoming of the current strain selection process is its heavy reliance on the HAI assay for antigenic characterization of surveillance isolates, which suffers several significant drawbacks. A final shortcoming is the inability to reliably predict whether rare antigenic variants will rise to prominence in nature during the vaccine production process, which results in poor vaccine match.

GoF approaches that lead to evasion of existing natural or induced immunity have potential to address all three shortcomings in the current strain selection process, through several different mechanisms.

Analysis of GoF approaches that improve strain selection capabilities by improving antigenic surveillance

As discussed above, GoF approaches have potential to strengthen the predictive value of molecular markers for antigenic drift and to improve the accuracy of existing models for predicting antigenic phenotype from genotype. Either strategy for sequence-based prediction of antigenic phenotype could be used to corroborate lab-generated HAI data in cases where results are difficult to interpret, thereby improving the quality of input data for the strain selection decision. Alternatively, sequence-based prediction methods could replace laboratory methods for antigenic characterization. Given that sequence data can be collected rapidly and economically and is increasingly being generated at NIC labs, reliance on sequence data may allow for consideration of a greater number of isolates, including isolates collected close to the VCM meeting dates. The result, an increase in the quantity of input data for the strain selection decision, would improve the process through a different mechanism. Critically, although molecular marker data informs strain selection decisions, neither molecular marker data nor predictive models are currently robust enough to replace phenotypic data (and may never be). Notably, GoF approaches are uniquely critical for advancing the state of the science for both approaches. Finally, full realization of these benefits requires continued expansion of sequencing capabilities at NICs, as only about one-quarter to one-half of HA sequences for seasonal flu strains are currently generated at NICs (depending on the influenza sub-type).[692]

Analysis of GoF approaches that improve strain selection capabilities through prediction of antigenic drift

GoF approaches to experimentally induce drift can be used to predict how circulating viruses may drift in nature, enabling production of vaccines against future, "drifted" strains that will antigenically match circulating viruses at their time of deployment. Specifically, the selection of antibody escape mutants of currently circulating viruses, through serial passaging or forward genetic screens conducted *in vitro* and *in vivo*, enables the identification of HA substitutions that confer escape. Coupled with genetic surveillance data, this information can be used to forecast the antigenicity of the next dominant strain to arise in nature.[693,694] However, whether and when such variants will emerge is uncertain, in part because stochastic events in natural evolution may result in the appearance of an unusual mutant that was not selected in the experimental studies. For that reason, this data is not currently incorporated into the strain selection process, and additional research is needed to determine whether it will be useful for predicting the course and timing of antigenic evolution in the future.[695]

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692 (2015w) Personal communication from WHOCC representative.

693 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

694 (2015l) Interviews with influenza researchers.

695 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

A different approach for predicting antigenic drift involves the use of computational models for antigenic evolution (though computational models could be used in conjunction with experimental data). Existing models cannot reliably predict antigenic drift, and two types of GoF studies are needed to improve the quality of existing models. First, a better understanding of the process of antigenic evolution will provide a foundation for the design of better models. As described above (Section 9.8.3.1.1), GoF approaches are uniquely capable of providing in-depth information about the evolutionary mechanisms driving antigenic drift as well as prospective information about the evolution of currently circulating viruses. Second, influenza modeling experts have stated that developing the ability to predict whether particular amino acid substitutions alter antigenicity in a given genetic context is critical for advancing the quality of these models.[696,697] As described in the preceding section, GoF approaches are essential for improving the accuracy of models for prediction of antigenic phenotype from genotype, although other types of data are also needed.

Taken together, utilizing experimental and/or *in silico* approaches to predict whether new antigenic variants are likely to emerge during the course of vaccine production would enable the production of vaccines based on those predicted future strains. This strategy would increase the likelihood that vaccines match the strains that are circulating during their target flu season, which will lead to an overall improvement in vaccine efficacy. One key concern associated with this strategy is that evolutionary predictions are difficult and are unlikely to be correct one hundred percent of the time, even as the science of prediction advances. Importantly, the exact amino acid sequence of the next dominant strain does *not* need to be predicted, but rather its antigenicity (as multiple sequences can fall into the same antigenic "cluster"). In addition, studies have shown that immunization with "antigenically advanced" vaccines (i.e., those that are based on predicted future strains) can provide some degree of protection against currently circulating strains.[698] Thus, even if the prediction is incorrect (i.e., the strain does not drift in nature), pre-emptive vaccination strategies are likely afford some degree of protection.

9.8.3.3.2 Vaccine Development Benefit 2: Development of Broad-Spectrum or Universal Flu Vaccines

Researchers are actively pursuing the development of broad-spectrum flu vaccines, which could protect against multiple strains (a subset of related strains within a subtype, an entire subtype, or multiple subtypes), and "universal" flu vaccines, which could protect against all strains. Either type of vaccine would eliminate the need for an exact match between vaccine strains and circulating seasonal viruses, thus improving the efficacy of seasonal flu vaccines. In addition, universal or broad-spectrum vaccines could be available rapidly during a pandemic or could be used to pre-vaccinate the population against emerging influenza strains, thereby increasing vaccine coverage during a pandemic. Scientists are exploring multiple strategies for development of such next-generation influenza vaccines, and both GoF and alt-GoF approaches have potential to inform this process.

Analysis of GoF approaches that inform the development of broad-spectrum or universal flu vaccines

GoF approaches that aim to map the antigenic landscape of the HA protein have potential to inform the development of broad-spectrum and universal influenza vaccines. Specifically, comprehensive forward genetic screens to identify which substitutions the HA protein can tolerate and which of those substitutions alter antigenicity will define the regions of the HA protein could drift (i.e., without significantly compromising the stability of HA and the viability of the virus) as well as how those regions can change antigenically. Defining all possible antigenic configurations of the HA protein provides a

696. Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382.
697. (2015) Interviews with influenza researchers.
698. Fonville JM *et al* (2014) Antibody landscapes after influenza virus infection or vaccination. *Science* 346: 996-1000.

foundation for developing a broad-spectrum vaccine (or vaccine cocktail) that protects against a large fraction of the possible antigenic space, thus pre-empting antigenic drift in nature and eliminating the need for annual production of seasonal flu vaccines.[699] Alternatively, defining those regions of the HA protein that do not mutate may provide a foundation for the development of a "drift-resistant" universal vaccine that targets those regions. Currently, whether either strategy will lead to the development of an effective influenza vaccine is unknown. In addition, comprehensive mapping of the antigenic landscape represents a labor-intensive, long-term project, and whether findings will be specific to an influenza strain or sub-type or will translate to other virus strains is unknown.

9.8.3.4 Benefits and limitations of GoF approaches to the development of therapeutics and diagnostics

GoF approaches in this phenotypic category are focused on elucidating mechanisms of antigenic drift in response to immune pressure, which is not relevant for the development of therapeutics or diagnostics. (We note that studies that generate escape mutants from candidate monoclonal antibody therapeutics, which are experimentally similar to approaches described above, are discussed in the "evasion of therapeutics" section.)

9.8.3.5 Benefits and Limitations of GoF Approaches to Policy Decisions

GoF approaches have potential to inform the selection of strains for the seasonal influenza vaccine in several ways, as described above.

9.8.3.6 Economic Benefits

GoF approaches that inform strain selection for seasonal influenza vaccines may improve the efficacy of seasonal flu vaccines by increasing the likelihood that the vaccine strains will match the strains that are circulating during the target influenza season. Ultimately, this benefit may increase vaccine uptake but otherwise is unlikely to yield economic benefits.

9.8.4 Identification of the Potential Benefits and Limitations of Alt-GoF Approaches

9.8.4.1 Benefits and Limitations of Alt-GoF Approaches to Scientific Knowledge

9.8.4.1.1 Scientific Knowledge Gap 1 – How Do Influenza Viruses Evolve Antigenically in Response to Immune Pressure?

The use of attenuated strains for serial passaging studies, in lieu of wild type strains, represents one type of alt-GoF approach for the study of antigenic evolution. Two types of attenuated strains are used for serial passaging studies to investigate antigenic evolution mechanisms: the mouse-adapted strain PR8, which is avirulent in people,[700] and 6:2R strains that contain the HA and NA gene segments from a seasonal strain of interest and the remaining six gene segments from PR8. While use of either type of attenuated strain can provide insight into the basic mechanisms of antigenic evolution, results may not translate to wild type strains due to differences in disease pathogenesis caused by wildtype versus attenuated strains as well as other factors. Moreover, 6:2R strains cannot be used to predict the effect of antigenic escape mutations on the fitness of wildtype strains because *in vivo* fitness is a complex, multi-genic trait that is highly dependent on genetic context. Finally, as the PR8 strain and 6:2R strains do not

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[699] (2015ii) Interview with Biomedical Advanced Research and Development Authority representative.

[700] Beare AS *et al* (1975) Trials in man with live recombinants made from A/PR/8/34 (H0 N1) and wild H3 N2 influenza viruses. *Lancet* 2: 729-732

efficiently infect ferrets,[701] these studies are limited to the use of mouse model systems, which is less representative of human disease than the ferret model system.

Comparative analysis of historical virus sequences that have drifted antigenically over time represents another alt-GoF approach for studying antigenic evolution. Relative to GoF approaches, the strength of the comparative sequence analysis approach is that it provides insight into the antigenic evolution of a wide breadth of influenza viruses in human populations. However, the success of this approach depends on the quality of available surveillance data; some strains have limited numbers of sequences available, and biases in the way that some surveillance data are collected render the data unsuitable or difficult to use. An additional limitation is that the historical record is static – that is, it cannot provide insight into mutations that were selected against, which is important knowledge for understanding the pressures and constraints that guide antigenic evolution. Finally, this approach cannot be used to proactively study the antigenic evolution of currently circulating viruses.

In silico approaches can be also used to investigate mechanisms underlying antigenic drift of influenza viruses. Existing models are largely based on and have been validated using historical data. As a result, the quality of these models is constrained by the set of limitations described above for the comparative sequence analysis approach. Although models can provide insight into the relationships between genetic and antigenic evolution, their accuracy in predicting future antigenic drift is unknown, thus any predictions must be experimentally validated.

9.8.4.1.2 Scientific Knowledge Gap 2 – What is the Molecular Basis of Antigenic Drift?

The use of attenuated reassortant strains containing the HA and NA genes from a seasonal strain of interest and the remaining six "internal" genes from the lab-adapted, attenuated strain PR8 (6:2R strains) in lieu of wild type strains represents one type of alternative approach for the study of the molecular basis of antigenic drift. Because the antigenicity of the HA protein is preserved in the context of a 6:2R strain,[702] 6:2R strains are as suitable as wild type strains for the discovery and confirmation of amino acid substitutions that lead to antigenic drift using *in vitro* or mouse model systems.

Several alternative experimental approaches can also be used to identify mutations associated with antigenic change. Comparatively analyzing the sequences of natural isolates that have drifted antigenically over time can lead to the identification of mutations that are associated with antigenic change. However, follow-up GoF experiments are needed to establish a causative link between particular mutations and antigenic change. Another drawback of this approach is that it is limited to the identification of amino acid substitutions that have arisen in nature, which represents a fraction of the possible antigenic space.

In silico approaches represent another alt-GoF approach for the identification of mutations associated with antigenic drift. Specifically, computational models based on antigenic, sequence, and HA structural data can be used to predict amino acid substitutions that will alter antigenicity. Although computational approaches can fully explore all possible antigenic configurations, existing models cannot predict mutations that will lead to antigenic change with certainty, thus the phenotypic consequences of any predicted mutation must be confirmed experimentally.[703]

Finally, the use of virus-like particles (VLPs) represents a virus-free alternative approach for testing whether particular mutations are *necessary* and *sufficient* to alter antigenicity in lieu of targeted genetic

[701] Jin H *et al* (2004) Imparting Temperature Sensitivity and Attenuation in Ferrets to A/Puerto Rico/8/34 Influenza Virus by Transferring the Genetic Signature for Temperature Sensitivity from Cold-Adapted A/Ann Arbor/6/60. *J Virol* 78: 995-998

[702] (2015l) Interviews with influenza researchers.

[703] (2015x) Influenza strain selection. Interview with industry personnel involved in vaccine production.

modification of wild type viruses. VLPs are virus-sized particles comprised of mammalian cell membrane studded with influenza HA and NA proteins but, as used for antigenic drift studies, do not contain other influenza proteins or influenza genetic material and are therefore non-infectious.[704,705] VLPs can be utilized in antigenic characterization assays in place of wild type viruses. Although the morphology – and, therefore, the antigenicity – of VLPs may differ slightly from that of whole viruses, influenza researchers stated that VLPs generally serve as good approximations for wild type viruses in antigenic characterization assays.[706]

9.8.4.1.3 Scientific Knowledge Gap 3 – What Are the Antigenic Sites on the HA Protein That Are Targeted by Neutralizing Antibodies?

Several alt-GoF approaches can also be used to map the antigenic epitopes of the influenza HA protein. One approach involves the use of cell surface display systems in yeast, bacteria, or bacteriophages. These systems exploit the ability of these organisms to express random peptides or protein fragments from the HA protein on their cell surface. Libraries of mutant bacteria/phages/yeast can then be screened for binding to a monoclonal antibody or post-infection sera, for mapping of the antigenic epitope of a particular antibody, or comprehensive mapping of antigenic sites, respectively. The main strength of this approach is that it is high-throughput, allowing for mapping of multiple antigenic sites at once through the use of complex sera or multiple mAbs. However, as the presentation of mapped epitopes may be different in the context of the full virus, GoF experiments with full virus should be performed to validate any findings.

Another alternative approach involves analysis of crystal structures of a viral protein (or protein fragment) complexed with a particular mAb. The crystal structure demonstrates precisely where an antibody binds to the HA protein, which can be compared to previous studies to determine whether the epitope is previously known or novel. The main drawback of this approach is that it is labor- and time-intensive and therefore has limited throughput. Additionally, researchers have faced technical limitations, such as difficulty crystallizing full-length HA proteins and radiation damage during the data collection process, which may compromise the quality of the data.[707]

Finally, targeted genetic modification of the HA protein using VLPs, a virus-free approach, can be used to confirm that particular amino acid substitutions are sufficient to confer escape from a particular neutralizing antibody, thereby suggesting that the mutated amino acids lie within the antibody binding site. Although influenza researchers stated that VLPs generally serve as good proxies for their cognate wild type viruses, one concern associated with this approach is that differences in the morphology of the VLP relative to the wild type virus may alter its antigenicity.

9.8.4.2 Benefits and Limitations of Alt-GoF Approaches to Surveillance

As described above, GoF approaches have the potential to benefit antigenic surveillance for human seasonal influenza viruses in two ways: (1) by improving the predictive value of molecular markers for antigenic drift and (2) by improving the accuracy of models for predicting antigenic phenotype from genotype. This section evaluates the ability of alternative experimental approaches to similarly strengthen

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704 Chen BJ *et al* (2007) Influenza virus hemagglutinin and neuraminidase, but not the matrix protein, are required for assembly and budding of plasmid-derived virus-like particles. *Journal of virology* 81: 7111-7123

705 Yu X *et al* (2008) Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 455: 532-536

706 (2015) Interviews with influenza researchers.

707 Hong M *et al* (2013) Antibody Recognition of the Pandemic H1N1 Influenza Virus Hemagglutinin Receptor Binding Site. *J Virol* 87: 12471-12480

the utility of molecular marker data and predictive models to understand whether alt-GoF approaches have the potential to benefit surveillance through either mechanism.

9.8.4.2.1 Analysis of Alt-GoF Approaches That Support the Use of Molecular Markers to Evaluate the Antigenicity of Seasonal Influenza Viruses

Currently, the predictive value of molecular markers for antigenic drift is limited by three sources of scientific uncertainty: (1) whether markers alter antigenicity in different genetic contexts, (2) whether novel amino acid substitutions at particular sites that are known to be associated with antigenic drift will alter antigenicity, and (3) what other amino acid substitutions confer antigenic change. Characterizing the antigenicity of wild type viruses that contain known molecular markers can demonstrate whether a known marker is associated with altered antigenicity in a new genetic context, but no alt-GoF approaches are capable of validating that the marker is necessary and sufficient to confer antigenic change in a new strain, which is essential for application of that knowledge to surveillance.[708] Similarly, characterization of wild type viruses is limited to determining whether different mutations at known sites or novel mutations are *associated* with antigenic change. Given the limited accuracy of existing models, predictions of any type must be experimentally confirmed using GoF approaches. However, in all cases attenuated reassortant strains can be used in lieu of wild type strains because the antigenicity of the 6:2R strain is similar to that of the parental wild type strain.

9.8.4.2.2 Analysis of Alt-GoF Approaches That Can Improve Predictive Models

Existing models for prediction of antigenic phenotype from genotype are largely built and validated using historical data. Though comparative analysis of additional historical sequences may uncover new amino acid substitutions that are associated with antigenic change, such data are unlikely to improve the ability of models to predict the antigenic phenotype of currently circulating viruses, which are evolving in new ways, and also cannot be used to validate those predictions. Thus, unlike GoF approaches, alt-GoF approaches are unable to substantially improve existing models by generating new experimental data about relationships between antigenic phenotype and genotype. However, several completely different types of data can increase the accuracy of these models and will complement improvements that can be gleaned through the use of GoF data. These additional data sources include crystal structures for the HA proteins from a wider variety of strains as well as data about how various amino acid substitutions affect HA stability, which can be generated using *in vitro*, virus-free approaches.[709]

9.8.4.3 Benefits and Limitations of Alt-GoF Approaches That Can Inform Vaccine Development

9.8.4.3.1 Vaccine Development Benefit 1: Improve Strain Selection Capabilities for Seasonal Influenza Vaccines

Alt-GoF approaches that have potential to benefit antigenic surveillance

GoF approaches have potential to benefit the strain selection process for seasonal influenza vaccines by improving methods for predicting antigenic phenotype based on genotype, namely the use of molecular markers of antigenic change and the use of computational models for sequence-based prediction of antigenicity. As described above, alt-GoF approaches have limited abilities to improve either method, relative to GoF approaches, though alt-GoF data complement GoF data to improve computational models.

[708] (2015i) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

[709] (2015l) Interviews with influenza researchers.

Alt-GoF approaches that have potential to inform predictions of antigenic drift

GoF approaches can also benefit the strain selection process by improving methods for predicting antigenic drift, which enables development of vaccines based on future, drifted strains, thereby increasing the likelihood the vaccines match circulating strains at their time of deployment. Comparative sequence analysis (alt-GoF) can also provide insight into antigenic evolution, which critically complements laboratory evolution studies by generating insights that are directly relevant to the evolution of flu viruses in human populations in nature. However, the ability of comparative sequence analysis to provide mechanistic information about evolution is severely limited relative to GoF approaches. In addition, analysis of wild type sequences cannot provide prospective information about the evolution of currently circulating viruses. For both reasons, the use of comparative sequence analysis approaches is not sufficient to improve the quality of existing models for antigenic evolution.

Alt-GoF approaches that have potential to improve strain selection capabilities through different mechanisms

Alternative strategies for improving the quality of antigenic characterization data upon which strain selection decisions are based are also being pursued. First, the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) aims to standardize methods for the HAI assay, which would ensure that antigenic data generated at disparate sites are more comparable.[710] A second effort to improve antigenic characterization data involves the development of alternative antigenic characterization assays, which have greater potential for standardization and automation than the HAI assay; however, alternative assays to date have had limited success.[711]

Several alternative approaches have potential to improve the strain selection process through completely different mechanisms. First, increasing the timeliness, representativeness, and availability of surveillance isolates would improve the accuracy of strain selection decisions by augmenting the quality of the input data upon which those decisions are based. Key elements of efforts to strengthen influenza surveillance systems include improving national surveillance systems, public health laboratories, and reporting and virus sharing procedures in developing countries.[712] To that end, between 2004 and 2014, the CDC invested more than $150 million toward building sustainable lab capacity and NICs and other international laboratories in over 40 less developed countries around the world.[713] The WHO and other WHO member countries also provide support in the form of funding, technical expertise, and guidance. However, given that resources for public health are limited and governments have many competing priorities, sustaining and building upon gains in these areas that have occurred in the wake of the 2009 pandemic will continue to pose a major challenge.[714,715]

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710 Van Kerkhove MD *et al* (2013) The consortium for the standardization of influenza seroepidemiology (CONSISE): a global partnership to standardize influenza seroepidemiology and develop influenza investigation protocols to inform public health policy. *Influenza Other Respir Viruses* 7: 231-234

711 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. *Vaccine* 33: 4368-4382

712 Ibid.

713 (2015p) Interview with Centers for Disease Control and Prevention representative.

714 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

715 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

Other lines of research and new technologies have potential to fundamentally change current influenza virological surveillance strategies and activities and may also lead to improved strain selection. For example, an improved understanding of the spatiotemporal distribution of viruses and the factors that influence the geographic spread of viruses could help target surveillance efforts and may also inform prediction of whether and when antigenic variants detected in a particular region are likely to arise.[716] Deep sequencing of surveillance isolates and systems biology approaches to analysis of such data may provide insight into the role of host-pathogen interactions in the antigenic evolution of viruses, which could also influence vaccination strategies and the strain selection process.[717] In these and other cases, because the state of the science and/or technology is preliminary, whether and when these approaches will have a demonstrated impact on strain selection for seasonal influenza vaccines is unknown.

Alt-GoF approaches that have potential to improve the efficacy of seasonal flu vaccines through different mechanisms

In addition to improving strain selection capabilities, several completely different strategies can be used to increase the efficacy of seasonal flu vaccines. These strategies are described in detail in Section 9.5.4.2.2 and are briefly summarized here. First, a universal or broad-spectrum flu vaccine would obviate the need for yearly production of strain-specific vaccines. However, influenza and vaccinology experts disagree about the scientific feasibility of developing a universal vaccine, and one expert felt that a ten to twenty year time frame for development is optimistic. Second, several scientific and technical advancements could shorten production timelines for strain-specific vaccines, which would enable strain selection closer to the start of flu season, presumably increasing the likelihood that the correct strains will be chosen. New vaccine platforms, such as recombinant vaccines, can be rapidly scaled up and have shorter production timelines than egg- and cell-based vaccines. However, the one recombinant vaccine on the market accounts for less than 1% of total seasonal influenza vaccine produced annually, and although several other virus-free vaccine platforms are in development, the length and expense of licensure processes for new vaccines will delay their widespread availability. Incorporating adjuvants into existing egg- and cell-based vaccines would allow for a smaller quantity of antigen to be used per vaccine dose, thus enabling production of the same number of doses in a shorter period of time. However, no US-licensed seasonal vaccines include adjuvants. Although an active area of research, adjuvanted vaccines must undergo standard FDA licensing procedures for new vaccines and thus are unlikely to be broadly available in the near future. Finally, GoF research that enhances virus production enables the development of higher-yield CVVs, which shortens vaccine production timelines by increasing the rate of bulk antigen production.

9.8.4.3.2 Vaccine Development Benefit 2: Inform Development of Universal or Broad-Spectrum Flu Vaccines

GoF approaches to define the antigenic landscape of the HA protein may inform the development of broad-spectrum or universal flu vaccines. Alternative approaches can also provide insight into which regions of HA mutate to alter antigenicity and the spectrum of antigenic configurations the HA protein can assume. First, attenuated reassortant strains (i.e., 6:2R strains with lab-adapted strains such as PR8) can be used for forward genetic screens in lieu of wild type strains. As the antigenicity of 6:2R strains is preserved relative to that of the parental seasonal flu strain, these strains are suitable for defining the landscape of antigenic configurations that are possible for the HA protein; however, it is possible that results may not translate to the wild type strain.

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716 Ibid.
717 Ibid.

Alternative experimental approaches can also be used to study the antigenic landscape of the HA protein. Comparative analysis of historical isolates can provide insight into mutations that are *associated* with antigenic drift over time. However, this approach is constrained to studying the fraction of antigenic space that the HA protein has explored in nature and cannot provide information about amino acid substitutions that compromise virus viability, which is important knowledge for mapping the suite of substitutions that are possible. Modeling approaches can, in principle, fully explore antigenic space but cannot yet accurately predict antigenic phenotype from genotype nor the effects of HA mutations on protein stability or viral fitness.

Completely different types of scientific data, generated through alt-GoF approaches, can also inform the development of universal and broad-spectrum influenza vaccines. For example, one method for identifying conserved epitopes involves identifying broadly neutralizing antibodies by characterizing the ability of different monoclonal antibodies to neutralize a variety of strains, followed by antibody epitope mapping.[718] This knowledge can inform the development of multiple vaccine types. Another method involves prediction of conserved immunogenic regions using *in silico* approaches, which has been used as a basis for the development of peptide-based vaccines.[719,720,721] Some of these vaccine candidates have been shown to be immunogenic in animal studies and Phase I clinical trials.[722,723,724] As all universal vaccines are in early stages of development, whether these approaches will prove to be successful in stimulating development of a safe, effective, and broad-spectrum influenza vaccine is unknown.

9.8.5 Comparison and Analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

9.8.5.1 Benefits to Scientific Knowledge

9.8.5.1.1 Scientific Knowledge Gap 1 – How Do Influenza Viruses Evolve Antigenically in Response to Immune Pressure?

GoF approaches are uniquely capable of providing in-depth information about the evolutionary mechanisms driving antigenic drift as well as prospective information about the evolution of currently circulating viruses. *In vivo* approaches provide insight into antigenic drift in response to selective pressure from the full complement of the immune system but may not translate to humans, while *in vitro* approaches can provide information about antigenic changes that arise in response to selective pressure from human antibodies but may not translate to complex, *in vivo* scenarios. In either case, lessons learned in the laboratory may not translate to virus behavior in human populations in nature. In contrast, comparative sequence analysis is uniquely capable of providing information about the antigenic evolution of viruses in nature, but is constrained to reactively studying the evolution of historic viruses in limited depth.

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718 Zhu X *et al* (2013b) A unique and conserved neutralization epitope in H5N1 influenza viruses identified by an antibody against the A/Goose/Guangdong/1/96 hemagglutinin. *J Virol* 87: 12619-12635

719 Gottlieb T, Ben-Yedidia T (2014) Epitope-based approaches to a universal influenza vaccine. *Journal of autoimmunity* 54: 15-20

720 Stoloff GA, Caparros-Wanderley W (2007) Synthetic multi-epitope peptides identified in silico induce protective immunity against multiple influenza serotypes. *European journal of immunology* 37: 2441-2449

721 Adar Y *et al* (2009) A universal epitope-based influenza vaccine and its efficacy against H5N1. *Vaccine* 27: 2099-2107

722 Ibid.

723 Pleguezuelos O *et al* (2012) Synthetic Influenza vaccine (FLU-v) stimulates cell mediated immunity in a double-blind, randomised, placebo-controlled Phase I trial. Ibid. 30: 4655-4660

724 Pleguezuelos O *et al* (2015) A Synthetic Influenza Virus Vaccine Induces a Cellular Immune Response That Correlates with Reduction in Symptomatology and Virus Shedding in a Randomized Phase Ib Live-Virus Challenge in Humans. *Clinical and vaccine immunology : CVI* 22: 828-835

9.8.5.1.2 Scientific Knowledge Gap 2 – What is the Molecular Basis of Antigenic Drift?

GoF approaches are uniquely capable of identifying amino acid substitutions that are *necessary* and *sufficient* to alter antigenicity in the context of whole viruses, which provides a critical foundation for follow-up studies to elucidate the biophysical basis of antigenic differences. Furthermore, GoF approaches represent the most efficient and reliable method for uncovering mutations that cause antigenic drift in circulating strains and are uniquely capable of exploring antigenic space to define which mutations do and do not lead to antigenic changes, which can improve predictive modeling efforts. For the purpose of discovering mutations that lead to antigenic change, GoF approaches can be conducted using attenuated 6:2R strains, instead of wild type strains, without compromising the quality and accuracy of the information that is generated. In addition, either 6:2R strains or VLPs can be used in lieu of wild type viruses to confirm that particular amino acid substitutions are necessary and sufficient to confer antigenic change, with the caveat that morphological differences between 6:2R strains or VLPs and their cognate wild type strains may lead to antigenic differences.

9.8.5.1.3 Scientific Knowledge Gap 3 – What Are the Antigenic Sites on the Ha Protein That Are Targeted by Neutralizing Antibodies?

Serial passaging of viruses in the presence of antibodies, a GoF approach, represents the only method for mapping the antigenic sites of the HA protein in the context of a full virus. However, the fact that mutations outside of antigenic sites may confer escape through long-range effects complicates interpretation of mutational data from these experiments. In addition, the approach is relatively low-throughput in that each passaging experiment enables identification of a single antigenic site, which is a drawback for experiments that aim to comprehensively map antigenic sites on the HA protein (but not for studies aiming to identify the recognition site of a particular mAb). In contrast, the use of cell surface display systems in yeast, bacteria, or phages represents a high-throughput method for identifying the antigenic sites of particular mAbs or for comprehensively mapping the antigenic sites on a given HA protein. Analysis of the crystal structures of HA-antibody complexes precisely reveals the antibody binding site, but the resources needed and technical challenges associated with this approach render it low-throughput. Confirming the results of an *in vitro* experiment requires determining whether mutating the proposed antigenic sites allows for escape from antibody neutralization, which can be done using whole viruses (GoF) or VLPs (alt-GoF). However, the relevance of all three *in vitro* approaches is limited by the fact that that HA presentation may differ in the context of the full virus.

9.8.5.2 Benefits to Surveillance

GoF approaches that lead to evasion of existing natural or induced immunity have potential to benefit surveillance of human seasonal influenza viruses in two ways: by increasing the utility of molecular markers for antigenic drift and by improving the accuracy of existing models for predicting antigenic phenotype from genotype. Attenuated reassortant strains (i.e., 6:2R strains with PR8) can be used in lieu of wild type strains without diminishing these benefits.

GoF approaches are uniquely capable of discovering new amino acid substitutions that are necessary and sufficient to alter antigenicity as well as determining whether markers are conserved in different strain contexts, which collectively increase the predictive value of molecular markers for antigenic change. Given the importance of genetic context for antigenic phenotype, whether such markers will ever be strongly predictive is as-yet-unknown. Notably, GoF approaches to define the antigenic plasticity of the HA protein are uniquely capable of addressing this question. Alternative experimental approaches cannot provide causative data on molecular markers that contribute to altered antigenicity and are limited to studying antigenic changes that have already occurred in nature, which significantly limits their utility for this application.

GoF approaches are uniquely capable of generating experimental data about novel mutations that are necessary and sufficient to confer antigenic change as well as validating predictions about antigenic phenotype based on the sequences of currently circulating viruses, which will improve the accuracy of existing predictive models. However, alternative types of data, including crystal structures of HA proteins from additional strains, are also needed to improve the quality of existing models and will complement gains achieved through the use of GoF approaches.

Together, molecular markers for antigenic change or predictive models can be used to supplement or replace the use of phenotypic assays for characterizing the antigenicity of circulating seasonal influenza viruses. Although molecular marker data currently informs the antigenic evaluation of surveillance isolates, neither molecular markers nor computational models are robust enough to replace phenotypic data (and may never be). However, use of these strategies to supplement phenotypic assays has potential to improve the quantity and quality of antigenic characterization data that can be considered during VCMs, which will increase the efficacy of seasonal flu vaccines as described below. Because molecular marker data are currently used to aid interpretation of surveillance data, new data can be seamlessly incorporated into the existing process, so that the only barrier to realization of this benefit is the need to strengthen the state of the science. Influenza researchers involved in the strain selection process stated that computational modeling could play an important role as well, once existing models are improved.[725] Notably, GoF benefits to the quantity timeliness of antigenic characterization data considered during VCM meetings rely on the generation of sequencing data at NICs (as opposed to WHOCCs). As less than half of HA sequences for seasonal flu viruses are currently generated at NICs, full realization of this benefit will necessitate further expansion of sequencing capabilities at NICs.[726]

9.8.5.3 Benefits to Vaccine Development

9.8.5.3.1 Vaccine Development Benefit 1: Improve Strain Selection Capabilities for Seasonal Influenza Vaccines

GoF approaches are uniquely capable of strengthening the predictive value of molecular markers for antigenic change and play a critical role in improving models for predicting antigenic phenotype from genotype as well as models for predicting antigenic drift. Although alternative experimental approaches can provide other types of data that also strengthen predictive models, these data complement rather than replace GoF data.

Advancing capabilities in these areas has the potential to benefit the strain selection process for seasonal influenza vaccines in several ways. First, using sequence-based prediction of antigenic phenotype to reinforce HAI assay results strengthens the robustness of antigenic characterization data, which provides a stronger foundation for strain selection decisions. Second, given that genetic surveillance data are increasingly available from NICs and other sample collection sites, shifting to sequence-based prediction of antigenic phenotype in lieu of laboratory assays has potential to increase the timeliness and quantity of surveillance data that are considered during VCMs. Third, predicting antigenic drift using models or through experimental GoF approaches would enable the development of antigenically advanced vaccines that are likely to match the circulating strains when vaccines are deployed, thereby increasing vaccine efficacy. However, full realization of these benefits necessitates further expanding sequencing capabilities at NICs.

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[725] (2015t) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

[726] (2015w) Personal communication from WHOCC representative.

Current experimental and modeling efforts cannot yet predict antigenic phenotype from genotype or the timing and direction of antigenic drift. Whether and when such capabilities will be sufficiently accurate to be incorporated into the strain selection process is unknown and depends both on scientific advancements and inherent features of influenza biology. Namely, the antigenic plasticity of the HA protein is not well-characterized but governs the feasibility of each of these predictive efforts. Notably, GoF efforts are also essential for advancing understanding of the antigenic landscape of HA.

Several alternative approaches have potential to improve the strain selection process through different mechanisms. First, efforts to standardize the HAI assay and to develop new antigenic characterization assays are ongoing, both of which have potential to improve the quality of antigenic characterization data. However, these alternative assays are not yet viable replacements for the HAI assay, and the degree to which increased standardization of the HAI assay will improve data quality is uncertain. Initiatives to strengthen global influenza surveillance systems have potential to improve the timeliness, representativeness, and quantity of surveillance isolates that can be considered at VCMs but face considerable funding and political barriers. Finally, new technologies such as deep sequencing have the potential to revolutionize influenza virological surveillance activities and may improve strain selection capabilities through unexpected mechanisms. Each of these alternative approaches either complements GoF approaches or addresses different shortcomings in the strain selection process.

Given the complexities involved in coordinating global influenza surveillance and making strain selection decisions under the time pressures imposed by vaccine production timelines, as well as the significant uncertainties in whether and when both GoF and alt-GoF approaches will yield demonstrable benefits to the process, pursuing both GoF and alt-GoF strategies in tandem will ensure that strain selection capabilities are advanced rapidly and to the greatest extent possible.

Finally, several alternative approaches have potential to improve the efficacy of seasonal influenza vaccines through completely different mechanisms. Universal vaccines represent the only strategy with potential to fully "solve" the vaccine mismatch problem but are in early stages of development and represent a long-term solution at best. Several approaches, namely the development of virus-free vaccines, the incorporation of adjuvants into existing vaccines, and the development of higher-yield vaccine viruses through GoF approaches that enhance virus production, have potential to shorten production timelines for strain-specific vaccines. This adjustment to manufacturing schedules could enable strain selection closer to the start of flu season, which presumably will increase the likelihood of vaccine match. Importantly, all of these approaches complement efforts to improve strain selection capabilities because each approach addresses different underlying gaps in current scientific and technical capabilities that contribute to vaccine mismatch. Thus, influenza vaccine experts recommend pursuing all of these approaches as part of comprehensive strategy for improving the quality of seasonal influenza vaccines.

9.8.5.3.2 Vaccine Development Benefit 2: Inform Development of Universal or Broad-Spectrum Flu Vaccines

GoF approaches are uniquely capable of defining the antigenic landscape of the influenza HA protein–that is, the spectrum of antigenic configurations that HA can assume and which regions of HA are capable of mutating while preserving virus viability. These data may inform the development of broad-spectrum influenza vaccines, which protect against a large fraction of the possible antigenic space, or universal influenza vaccines, which target regions of the protein that are unable to mutate and thus are drift-resistant. Alternative experimental approaches have significant limitations. Attenuated reassortant strains can be used to explore possible antigenic configurations, but results regarding the fitness consequences of mutations may not translate to wild type strains. Comparative analysis of historical isolates is limited to the fraction of antigenic space that has been explored in nature and cannot provide information on

mutations that compromise virus viability. While virus-free approaches can be used to explore new antigenic space, these approaches do not reveal the fitness consequences of mutations either. Finally, existing models cannot accurately predict antigenic phenotype from genotype or predict the fitness consequences of particular mutations.

Mapping the antigenic landscape of the HA protein represents a labor-intensive project, and whether vaccine development strategies based on the information gleaned from this approach will be successful is unknown. Other strategies for developing broad-spectrum and universal vaccines, such as *in silico* prediction of conserved epitopes for the development of peptide-based vaccines, have shown promise. All universal/broad-spectrum vaccine candidates are in early stages of development, and which strategy is likely to be most successful is unknown. Given the challenges for developing universal/broad-spectrum vaccines, pursuing all experimental approaches that support vaccine development in tandem, including GoF approaches, will maximize the likelihood of success, which could have large public health impacts.

9.9 Influenza Viruses: Benefits of GoF Research That Leads to Evasion of Vaccines

9.9.1 Summary

This section describes the benefits of GoF research that is reasonably anticipated to lead to evasion of influenza vaccines in development. Such GoF studies were found to have unique benefits to the development of new influenza vaccines. No alternative approaches were identified that can provide similar benefits. Chapter 9.9 provides an overview of these benefits, including basic background and Supporting Information; a fully referenced and more thorough discussion of these benefits can be found in Appendix IV Section 15.6.

9.9.1.1 Benefits of GoF That Leads to Evasion of Vaccines to Vaccine Development

- GoF approaches are uniquely capable of determining whether and how readily influenza viruses can acquire mutations to escape neutralization by candidate broad-spectrum or universal influenza vaccines, a critical aspect of testing the potential field efficacy of vaccine candidates.
- There are no alt-GoF approaches that can provide similar information.

9.9.2 Overview of GoF Research Landscape: Evasion of Vaccines

9.9.2.1 Serial Passaging of Viruses in the Presence of Post-Vaccination Sera

Serial passaging of a virus in cells in the presence of animal sera produced in response to a vaccine or in vaccinated animals may lead to the emergence of viruses that are resistant to neutralization by vaccine-induced antibodies. This approach is used to test whether and how readily viruses can evolve to evade vaccines in development, for example new vaccine platforms that are more broad-spectrum or resistant to drift than current influenza vaccine platforms, which is an important indicator of the potential field efficacy of the vaccine. Most of these experiments involve next-generation influenza vaccine candidates targeting epitopes other than the globular head domain of the hemagglutinin (HA) protein, the target of current influenza vaccines. Given that the globular head domain of HA is the immunodominant protein of influenza viruses and that these next-generation vaccines are not yet widely available, strains that can overcome the protection afforded by these vaccines are expected to pose a minimal increase in human health risk relative to wild type strains.

Because seasonal influenza vaccines are updated annually, approaches that lead to the generation of vaccine strains that are no longer neutralized by vaccine-induced antibodies are more appropriately described by the "evasion of existing induced immunity" phenotype. In addition, we did not identify any studies involving H5N1 viruses that would be expected to lead to the generation of viruses that cannot be neutralized by the pre-pandemic H5N1 vaccine in the national stockpile.

9.9.3 Identification of the Potential Benefits and Limitations of GoF Approaches

In this section, the potential benefits of GoF research that leads to evasion of vaccines in each benefit category listed in the NSABB Framework are discussed.

This GoF approach is solely focused on understanding how a virus evolves in response to immune pressure from a vaccine under development. As a result, insights gleaned from this approach do not benefit scientific knowledge, surveillance or policy decisions (because the vaccine has not yet been deployed) or the development of therapeutics and diagnostics.

9.9.3.1 Benefits and Limitations of GoF Approaches to Vaccine Development

GoF approaches that lead to evasion of vaccines in development benefit the development of new influenza vaccines. Specifically, these approaches demonstrate whether and how readily viruses can drift to escape neutralization by new vaccine candidates, which is an important indicator of their potential field efficacy relative to existing vaccines.

9.9.3.1.1 Shortcomings in Existing Influenza Vaccines

Because existing influenza vaccines are strain-specific, new seasonal flu vaccines must be produced annually in order to accommodate antigenic drift of circulating influenza viruses, and new pandemic flu vaccines must be produced in response to the emergence of a novel pandemic strain. The long production timelines for existing influenza vaccines critically limit the mitigating impact of influenza vaccination on the morbidity and mortality associated with influenza outbreaks, as discussed in Section 9.5.3.3.1. For these reasons, the influenza research and public health communities are strongly interested in developing a broad-spectrum or universal flu vaccine.[727,728] Demonstrating whether such vaccine candidates are more resistant to antigenic drift than existing vaccines is a critical aspect of testing the potential field efficacy of these vaccine candidates.[729]

9.9.3.1.2 Benefits and Limitations of GoF Approaches

Serial passaging of viruses in cells, in the presence of sera from vaccinated animals, or in vaccinated animals may lead to the emergence of mutant viruses that can no longer be neutralized by vaccine-induced antibodies. Sequencing of emergent escape mutants provides insight into how readily viruses can acquire mutations that confer escape from protective vaccination (i.e., how many mutations are needed to escape neutralization). Follow-up studies characterizing other properties of emergent escape viruses relative to the parental virus, such as fitness, may provide additional insight into how likely vaccine escape mutants are to emerge and persist in human populations. *In vitro* studies provide a proof of principle demonstration of whether viruses can mutate to escape vaccines, but virus behavior in response to relatively simple selection pressures may not translate to human populations. *In vivo* studies involve

[727] Rudolph W, Ben Yedidia T (2011) A universal influenza vaccine: where are we in the pursuit of this "Holy Grail"? *Human vaccines* 7: 10-11

[728] (2015) Interviews with influenza researchers.

[729] Ibid.

complex selection pressures that more closely mimic those that a virus will encounter during infection of a vaccinated human host, but results in representative animal models may not translate to human disease.

9.9.3.2 Economic Benefits of GoF Approaches

GoF benefits to the development of new vaccines may have downstream economic benefits. Economic benefits were not explicitly evaluated in this report.

9.9.4 Identification of the Potential Benefits and Limitations of Alt-GoF Approaches That Provide Similar Potential Benefits to the GoF Approaches Being Examined

No alternative approaches are capable of evaluating whether viruses can acquire mutations to escape neutralization by candidate vaccines prior to field deployment of the vaccine.

9.9.5 Comparison and Analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

Taken together, GoF approaches are uniquely capable of determining whether and how readily influenza viruses can acquire mutations to escape neutralization by candidate broad-spectrum or universal influenza vaccines, a critical aspect of testing the potential field efficacy of vaccine candidates.

9.10 Influenza Viruses: Benefits of GoF Research That Leads to Evasion of Therapeutics

9.10.1 Summary

This section describes the benefits of GoF research that is reasonably anticipated to lead to evasion of therapeutics, including licensed therapeutics and therapeutics in development. Such GoF studies were found to generate scientific knowledge to inform surveillance of circulating seasonal and animal influenza viruses, which guides therapeutic recommendations for seasonal flu and decision-making about pandemic preparedness initiatives, respectively; to benefit the production of influenza vaccines; and to benefit the development of new therapeutics. Alt-GoF approaches that may generate similar benefits were also identified and analyzed. At present, GoF studies resulting in evasion of existing natural or induced adaptive immunity have unique benefits to scientific knowledge, surveillance, and therapeutic development, though full realization of GoF benefits to surveillance requires scientific advancements and expansion of global public health surveillance networks. Chapter 9.10 provides an overview of these benefits, including basic background and Supporting Information; a fully referenced and more thorough discussion of these benefits can be found in Appendix IV Section 15.7.

9.10.1.1 Benefits of GoF Research That Leads to Evasion of Therapeutics to Scientific Knowledge

- GoF approaches:
  - Are the most efficient and effective strategies for discovering novel mutations that confer resistance to antivirals.
  - Are uniquely capable of identifying mutations that are *necessary* and *sufficient* to confer antiviral resistance across multiple strain contexts, which provides a strong foundation for follow-up studies to elucidate the mechanisms underlying antiviral resistance.

- Attenuated reassortant strains may be used in lieu of wild type strains for many experiments investigating the mechanistic basis of resistance, but results may not be recapitulated in the context of the wild type viruses.

- Are the most efficient and effective strategy for gaining in-depth insight into the viral and host selection pressures that shape the emergence and spread of antiviral resistance.

- Alt-GoF approaches:
  - Are capable of discovering novel mutations associated with antiviral resistance but have limitations relative to GoF approaches.

  - Equally capable of establishing a causal link between a particular mutation and antiviral resistance, but are limited in their ability to demonstrate that the function of markers is conserved across strain contexts by the breadth of antiviral resistant strains that exist in nature.

  - Comparative analysis of patient isolates over the course of antiviral treatment can provide in-depth insight into the evolution of antiviral resistance, but such studies are relatively rare and may not translate to the general population.

  - Other alt-GoF approaches provide limited mechanistic insight about the evolutionary pressures driving emergence of antiviral resistance.

9.10.1.2 Benefits of GoF Research That Leads to Evasion of Therapeutics to Surveillance

- GoF approaches:
  - Provide unique benefits for strengthening the predictive value of molecular markers for antiviral resistance, thereby improving their utility for interpreting surveillance data.

  - Molecular markers (discovered and validated through GoF approaches) have potential to strengthen the quality and timeliness of antiviral resistance information about viruses collected through surveillance, by:
    - Corroborating phenotypic characterization data, and
    - Enabling sequence-based prediction of the antiviral resistance phenotype, prior to the availability of phenotypic data.

  - Full realization of the benefits of GoF approaches to surveillance is subject to expansion of sequencing capabilities at public health laboratories that collect clinical specimens.

- Alt-GoF approaches:
  - Are significantly limited in their ability to strengthen the predictive value of molecular markers for antiviral resistance.

  - Phenotypic assays play a critical role in evaluating the antiviral resistance of surveillance isolates because they provide direct information about the degree of antiviral resistance of a particular strain, so that sequence-based predictions should be confirmed whenever possible.

9.10.1.3 Benefits of GoF Research That Leads to Evasion of Therapeutics to Decision-Making in Public Health Policy

- GoF approaches:

- Data on the prevalence of antiviral-resistant seasonal strains, collected through surveillance, informs therapeutic recommendations developed by the CDC. Both molecular marker data (GoF) and phenotypic data (alt-GoF) inform interpretation of surveillance data.

- Improving the practice of using molecular marker (GoF) may enable a larger quantity of strains to be assessed for their antiviral sensitivity, which would improve the ability to detect and track the emergence of rare antiviral-resistant strains.

- The observation of antiviral resistance in an animal influenza strain, coupled to other factors indicative of increased pandemic potential, may trigger downstream responses such as applying for Emergency Use Authorization (EUA) for antivirals in development.
  - Using molecular markers for antiviral resistance (GoF) enables a rapid risk assessment based on sequence data when a novel virus first emerges in human populations, which can provide a several week head start on downstream response activities.

- Alt-GoF approaches:
  - Results from phenotypic testing of seasonal flu surveillance isolates critically informs therapeutic guidelines for seasonal flu. A subset of surveillance isolates are subjected to phenotypic testing for antiviral resistance.

  - Confirming whether an animal influenza strain is antiviral-resistant through phenotypic testing is critical for pandemic risk assessments and downstream decision-making, but results from phenotypic assays may be delayed relative to the publication of sequence data due to delays in shipping of virus samples.

9.10.1.4 Benefits of GoF Research That Leads to Evasion of Therapeutics to Vaccine Development

- GoF approaches:
  - Are the most efficient and effective way to discover novel markers for antiviral resistance and can establish a causal link between a particular mutation and antiviral resistance across many strain contexts. These conserved markers can be mutated out of vaccine viruses to increase the safety of the vaccine production process.

- Alt-GoF approaches:
  - Can be used to establish a causal link between a particular trait and antiviral resistance, but their ability to demonstrate that a particular marker is conserved across strain contexts is limited by the breadth of antiviral resistant strains that exist in nature.

9.10.1.5 Benefits of GoF Research That Leads to Evasion of Therapeutics to Therapeutic Development

- GoF approaches:
  - Are uniquely capable of screening potential therapeutic candidates based on how readily antiviral resistance emerges, which is an important indicator of the potential field efficacy of a therapeutic.

  - Are uniquely capable of determining whether the acquisition of resistance to a therapeutic candidate increases the infectivity, transmissibility, or virulence of a virus, which is an important aspect of safety testing of the therapeutic candidate.

  - Are uniquely capable of determining the genetic threshold for resistance to a new therapeutic, prior to field deployment of that therapeutic.

- Are uniquely capable of identifying the viral target of a novel therapeutic with an unknown mechanism of action, which is valuable for determining the mechanism of action of therapeutic candidates identified through unbiased high-throughput screens.

- Are uniquely capable of determining the therapeutic dose that is least likely to lead to the acquisition of antiviral resistance as well as determining whether combination therapies better prevent the emergence of resistant viruses than individual therapies, which informs the development of therapeutic strategies that will be effective for a longer time in the field.

- Alt-GoF approaches:
  - X-ray crystallography and photoaffinity crosslinking are limited to the study of therapeutics with known viral targets, and inferring mechanistic information based on static data about drug-viral interactions may be difficult.

  - RNAi screens to identify host factors that are required for the antiviral activity of a therapeutic provide indirect information about the mechanisms of therapeutics that target viral proteins.

9.10.2 Overview of GoF Research Landscape: Evasion of Therapeutics

9.10.2.1 Serial Passaging of Viruses in the Presence of Therapeutics

Serial passaging of viruses in the presence of a therapeutic may lead to the acquisition of mutations that allow the virus to evade inhibition by the therapeutic. This approach is performed to determine whether and how readily a virus evolves resistance in response to selective pressure from a therapeutic and to identify mutations that confer resistance, which provides a foundation for follow-up studies investigating the mechanism of action of the therapeutic and the mechanistic basis of antiviral resistance. When passaging experiments are performed using a new therapeutic candidate with an unknown viral target, this information also helps to identify the therapeutic target, as resistance mutations are most likely to arise in the target protein. Of note, the acquisition of resistance to novel classes of therapeutics is not expected to confer cross-resistance to existing antivirals (i.e., adamantanes or neuraminidase inhibitors). Thus, when these experiments involve drug candidates within new classes of therapeutics, which are not yet widely available, no increase in human health risk is posed by resistant strains. Serial passaging approaches have been performed using cell culture, animal models, and (rarely) human challenge experiments.

9.10.2.2 Forward Genetic Screen to Identify Mutations That Confer Antiviral Resistance

Forward genetic screens involve random mutagenesis of antiviral target proteins (e.g., the influenza neuraminidase protein) followed by screening of mutants to identify those with reduced antiviral susceptibility (e.g., to NAIs). Follow-up studies may determine the consequences of antiviral resistance mutations on other virus phenotypes, such as viral fitness. As for serial passaging experiments, the identification of mutations that confer antiviral resistance provides a foundation for studies to elucidate antiviral resistance mechanisms.

9.10.2.3 Targeted Modification of Viruses to Introduce Mutations That Are Expected to Lead to Evasion of Therapeutics

A second approach involves targeted genetic modification of a virus to introduce mutations that are associated with antiviral resistance, which may have been identified through GoF approaches such as serial passaging or through alt-GoF approaches such as comparative analysis of sequences from patients

who did and did not respond to antiviral treatment. This experiment serves to demonstrate that a particular mutation or set of mutations is necessary and sufficient to enhance antiviral resistance, which provides a foundation for follow-up studies investigating the mechanistic basis of antiviral resistance.

9.10.3 Identification of the Potential Benefits and Limitations of GoF Approaches

9.10.3.1 Benefits and Limitations of GoF Approaches to Scientific Knowledge

Only one class of licensed antivirals are recommended for therapeutic use against seasonal influenza viruses: the neuraminidase inhibitors (NAIs), which inhibit the activity of the NA protein.[730,731,732] Although most circulating strains have been sensitive to all three licensed NAIs during recent flu seasons, strains that are resistant to one or more NAIs have been observed in nature in A/H1N1,[733] A/H3N2,[734] and B strains.[735] Resistance has been linked to a variety of mutations, and in most cases, the mechanisms underlying drug resistance are not well understood. In addition, the factors that shape whether resistant strains will emerge, spread and persist in human populations, including the contribution of viral factors such as the relative fitness of resistant strains, are unknown.

In this section, the ability of GoF approaches, versus alternative experimental approaches, to address two unanswered questions in this field are addressed:

- What are the genetic traits underlying resistance to NAIs, and what is the mechanistic basis of resistance?
- What selection pressures shape whether and how readily antiviral-resistant strains arise and spread in nature?

9.10.3.1.1 Scientific Knowledge Gap 1: What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?

Serial passaging of viruses in the presence of one or multiple therapeutics may lead to the emergence of viruses that are resistant to inhibition by the therapeutic. Sequencing emergent antiviral-resistant viruses enables the identification of novel mutations that are sufficient to confer resistance. Selection for resistance studies can be carried out *in vitro*, *in vivo*, in animals, or through human challenge experiments. (Human challenge experiments are rare and have only been conducted using human seasonal strains.) Notably, *in vitro* and *in vivo* selection approaches equally enable the identification of mutations associated with antiviral resistance, though the *in vitro* approach is faster and cheaper. The *in vitro* approach is highly efficient and can be carried out using any virus strain, including currently circulating strains. Importantly, as multiple mutations may arise during passaging, follow-up studies may be needed to determine which mutation(s) are responsible for the antigenic escape phenotype.

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730 CDC. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Update November 3, 2015. Accessed November 28, 2015.

731 Kim CU *et al* (1997) Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J Am Chem Soc* 119: 681-690

732 Li W *et al* (1998) Identification of GS 4104 as an orally bioavailable prodrug of the influenza virus neuraminidase inhibitor GS 4071. *Antimicrob Agents Chemother* 42: 647-653

733 Gubareva LV *et al* (2001) Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *J Infect Dis* 183: 523-531

734 Abed Y *et al* (2006) Impact of neuraminidase mutations conferring influenza resistance to neuraminidase inhibitors in the N1 and N2 genetic backgrounds. *Antiviral therapy* 11: 971-976

735 Fujisaki S *et al* (2012) A single E105K mutation far from the active site of influenza B virus neuraminidase contributes to reduced susceptibility to multiple neuraminidase-inhibitor drugs. *Biochem Biophys Res Commun* 429: 51-56

Forward genetic screens, which involve random mutagenesis of the NA genes from antiviral-sensitive strains followed by screening of mutants to identify those with reduced antiviral susceptibility, represent another GoF approach for discovering novel mutations that confer antiviral resistance. The screening approach is less efficient that the selection approach but may enable the discovery of rare antiviral resistance mutations that might be out-competed during a selection experiment due to fitness defects. Depending on the mutagenesis strategy used, follow-up studies may be needed to determine which mutation(s) are responsible for the antiviral resistance phenotype. Additionally, for both the serial passaging and forward genetic screen approaches, results may not translate to other strain contexts.

Targeted genetic modification of parental viruses to introduce mutations associated with antiviral resistance, followed by phenotypic characterization of the antiviral sensitivity of mutant viruses, is used to demonstrate that a mutation or set of mutations is necessary and sufficient to confer resistance. Subsequently, to determine whether the phenotypic consequences of mutations are functionally generalizable across multiple virus strains, targeted mutagenesis can be used to introduce mutations into new virus strains, followed by characterization of antiviral sensitivity. Together, these results provide a strong foundation for follow-up biochemical, cell biological, structural, and other studies to determine the mechanistic basis of antiviral resistance.

9.10.3.1.2 Scientific Knowledge Gap 2: What Selection Pressures Shape Whether and How Readily Antiviral-Resistant Strains Arise and Spread in Nature?

Serial passaging of viruses in the presence of one or more therapeutics to select for antiviral-resistant strains provides insight into whether and how readily antiviral resistance arises. Follow-up experiments to characterize other phenotypic properties of emergent resistant viruses, such as fitness, virulence, and transmissibility, may provide insight into how likely resistant viruses are to emerge, spread, and persist in human populations. These experiments have been conducted *in vitro* and *in vivo*, through animal experiments and human challenge experiments. Due to the simple selection pressures encountered by viruses during passage in cell culture, the *in vitro* approach is less useful than the *in vivo* approach for understanding how selection pressures in human populations are likely to drive the emergence and spread of antiviral-resistant viruses. The ability to gain direct insight into emergence of resistance in humans through human challenge experiments is valuable, but ethical considerations severely constrain the number and scope of experiments that can be carried out. Animal experiments provide a controlled system for studying the emergence of resistance under complex selection pressures, including identifying resistance mutations that arise but are negatively selected within or between hosts. However, results in animal models may not translate to human populations.

9.10.3.2 Benefits and Limitations of GoF Approaches to Surveillance

Through influenza surveillance, public health professionals monitor the appearance and prevalence of NAI-resistant strains of seasonal influenza viruses circulating in global populations and of animal influenza viruses that have caused human infections. Data on the prevalence of antiviral-resistant seasonal strains informs therapeutic recommendations developed by the CDC (i.e., which of the three FDA-approved NAIs should be recommended for treatment).[736] In the context of surveillance for zoonotic influenza infections in humans, data on antiviral resistance informs decision-making about pandemic preparedness initiatives because antiviral resistance is one of the risk elements considered in a pandemic risk assessment.

[736] Centers for Disease Control and Prevention. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Accessed: November 4, 2015

Resistance to NAIs can be assessed in two ways: through laboratory testing of NAI resistance using the fluorometric 20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid (MUNANA) assay or other assays and/or by inspecting sequences for the presence of mutations that are known to confer NAI resistance ("molecular markers"). This section evaluates the potential for GoF approaches to improve the practice of using molecular markers for antiviral resistance and the relative utility of sequence-based predictions versus phenotypic assays for surveillance for antiviral resistant viruses. The public health actions that are taken downstream of this surveillance are described in the "informing policy decisions" section below.

9.10.3.2.1 Benefits of Using Molecular Markers for Antiviral Resistance to Infer Antiviral Resistance Phenotype from Genotype

The practice of using molecular markers for antiviral resistance to predict the antiviral resistance phenotype of viruses detected through surveillance provides several advantages relative to the use of phenotypic assays. In particular, the ability to sequence clinical samples is valuable because the composition of viral quasispecies changes during the virus isolation process, which can mask the presence of antiviral resistant strains in mixed infections if those resistant strains are lost during the virus isolation process.[737] In addition, the expansion of viral sequencing capabilities at the "base" level of the surveillance system (NICs and other diagnostic labs) means that sequencing data are increasingly available prior to phenotypic data, which are generated at WHOCCs.[738] Thus, the use of molecular markers can enable a more timely assessment of the antiviral resistance phenotype of strains detected through surveillance. Finally, as sequencing becomes cheaper and easier, the number of surveillance isolates that are sequenced is likely to increase, such that using molecular markers will enable consideration of a larger number of viruses than can be subjected to phenotypic characterization assays.[739]

9.10.3.2.2 Current Utility and Shortcomings of Using Molecular Markers for Antiviral Resistance

NAI resistance can arise from one or two mutations, and many mutations have been identified that confer resistance to one or multiple NAIs. Several markers for NAI resistance have been shown to be functionally generalizable, conferring resistance in multiple strain contexts.[740] In the experience of influenza researchers and government officials involved in surveillance, the presence of such a validated antiviral resistance marker is strongly predictive antiviral resistance. However, the absence of a known resistance marker is not necessarily predictive of antiviral sensitivity, as it is likely that additional mutations can lead to resistance. This lack of knowledge about the mutational landscape that permits evolution of antiviral resistance limits the current utility of sequence-based approaches for predicting resistance. Moreover, validating known markers in additional strain contexts will further strengthen their predictive value. As discussed in detail in Section 9.10.3.1.1 above, both GoF and alt-GoF approaches can provide insight into these scientific questions. The relevant findings are summarized here.

9.10.3.2.3 Potential Benefits of GoF Approaches to the Practice of Using Molecular Markers for Antiviral Resistance

GoF approaches represent the most efficient and effective strategy for discovering novel mutations that give rise to antiviral resistance and are uniquely capable of confirming that particular mutations are *necessary* and *sufficient* to confer resistance in multiple strain contexts. Notably, for mutations that confer resistance by altering the function of the NA protein (i.e., versus altering NA expression levels or through epistatic effects), these experiments can be performed using attenuated reassortant strains, though results

737 (2015j) Interviews with CDC and BARDA representatives.
738 Ibid.
739 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.
740 Boivin G (2013) Detection and management of antiviral resistance for influenza viruses. *Influenza and Other Respiratory Viruses* 7: 18-23

may not be recapitulated in the context of the wild type strain. Taken together, these approaches strengthen the predictive value of molecular markers for antiviral resistance, thereby improving their utility for interpreting surveillance data.

9.10.3.3 Benefits and Limitations of GoF Approaches to Decision-Making in Public Health Policy

GoF approaches have potential to benefit surveillance for antiviral resistant strains by improving the practice of using molecular markers for antiviral resistance to infer antiviral resistance from genotype. Surveillance for antiviral resistant strains informs downstream decision-making related to public health practice and policy. Namely, data on the prevalence of antiviral-resistant seasonal strains informs therapeutic recommendations developed by the CDC, and antiviral resistance is one of the risk elements considered in a pandemic risk assessment of an animal influenza virus. This section describes each of these applications, which illustrate the ultimate public health impacts associated with GoF benefits to surveillance.

9.10.3.3.1 Benefits to Decision-Making Related to Seasonal Flu Strains

The CDC monitors the prevalence of antiviral resistance among circulating strains to inform their recommendations to clinicians for the use of influenza antivirals. Although recent seasonal outbreak strains have remained susceptible to all three NAIs, sporadic cases of antiviral resistant viruses continue to be detected.[741] Current antiviral treatment guidelines do not recommend particular NAIs; however, an increase in the prevalence of singly-resistant strains could trigger a recommendation change. As antivirals are most effective when given within 48 hours of symptom onset, the CDC recommends initiating antiviral treatment prior to laboratory confirmation of influenza (i.e., without knowledge of antiviral susceptibility).[742] Given that, antiviral treatment recommendations based on reliable knowledge about the prevalence of resistance to particular antivirals among circulating strains are essential for the success of therapeutic treatment. Currently, a subset of the influenza viruses that are collected by WHOCCs are sent to CDC for antiviral susceptibility testing.[743] As discussed above, phenotypic assay results are often corroborated by inspection of sequences for the presence of molecular markers associated with antiviral resistance, and the use of molecular markers may expand the number of viruses that can be evaluated for their antiviral susceptibility as the number of surveillance isolates that are sequenced increases. This increase will provide a stronger foundation for antiviral treatment recommendations and may enhance detection of rare antiviral-resistant variants to increase awareness.

9.10.3.3.2 Benefits to Decision-Making Related to Pandemic Influenza

Antiviral resistance is one of the risk elements considered in pandemic risk assessments of animal influenza viruses, which inform downstream decision-making about investments in pre-pandemic preparedness initiatives (discussed in detail in Section 9.6.3.3.2 and Section 9.6.3.3.3). The antiviral resistance risk element does not contribute to the likelihood that an animal virus will emerge to efficiently infect and transmit in humans and moderately contributes to the assessment of the expected consequences of an emergence event. For example, in a recent risk assessment of avian H7N9, avian H1N1, and swine H3N2v viruses, the antiviral resistance element was worth approximately one-third as much as the most highly weighted disease severity element.[744] Stakeholders involved in the pandemic risk assessment

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741 Centers for Disease Control and Prevention. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Accessed: November 4, 2015

742 Ibid.

743 Centers for Disease Control and Prevention. Antiviral Drug Resistance among Influenza Viruses. http://www.cdc.gov/flu/professionals/antivirals/antiviral-drug-resistance.htm. Last Accessed: November 4, 2015

744 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

process emphasized that antiviral resistance does not increase risk a priori but rather is important when coupled to other factors indicative of increased pandemic potential, such as a high number of human infections or enhanced transmissibility or virulence in ferrets. In this case, the observation of antiviral resistance may trigger USG representatives to initiate the process of applying for Emergency Use Authorization (EUA) from the FDA for antivirals in development, to ensure that effective antivirals will be available if the strain under evaluation spreads to cause a pandemic. When evaluating antiviral resistance, stakeholders consider both phenotypic and genetic data when possible but noted that the ability to conduct a rapid risk assessment using molecular markers is valuable when strains first emerge and sequences are published prior to the receipt of viral isolates. This rapid assessment can provide a several week head start on the EUA process. As EUAs may be issued within days following a request if the FDA has worked with government partners on a "pre-EUA" package, this head start could significantly impact the timing of availability of antivirals in the event of a pandemic.[745,746]

9.10.3.4 Benefits and Limitations of GoF Approaches to the Development of Vaccines

9.10.3.4.1 Vaccine Development Benefit 1: Targeted Mutagenesis to Remove Antiviral Resistance Markers from Vaccine Viruses

Vaccine viruses comprise the HA and NA genes from the wild type strain of interest and the remaining six genes from a vaccine backbone virus such as PR8. Mutations that confer resistance to NAIs, the one approved class of influenza antivirals that are recommended for use in the US, arise in the NA gene.[747,748,749,750] If the wild type NA gene contains conserved markers for NAI resistance, these markers can be removed through targeted deletion or mutagenesis to increase the safety of the vaccine production process. (Of note, most influenza vaccines produced in the US are inactivated, thus whether a vaccine strain is sensitive or resistant to antivirals has no impact on the safety of the vaccine itself.)

GoF approaches represent efficient and effective strategies for the discovery of new antiviral resistance markers but may uncover mutations that do not yet exist in nature, which is not relevant for this application because vaccine viruses are based on wild type viruses. Subsequently, targeted mutagenesis of antiviral-sensitive strains to introduce mutations expected to confer antiviral resistance, can be used to demonstrate that a mutation or set of mutations is necessary and sufficient to confer antiviral resistance. The establishment of such a causal link is critical for the application of this information to vaccine development.

9.10.3.5 Benefits and Limitations of GoF Approaches to the Development of Therapeutics

Given that only one class of antivirals is licensed and recommended for use in the US and strains that are resistant to one or more therapeutics in this class have been detected in nature, there is an urgent need for the development of new therapeutics against influenza viruses.[751]

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745 Administration FaD. Guidance - Emergency Use Authorization of Medical Products. http://www.fda.gov/RegulatoryInformation/Guidances/ucm125127.htm. Last Update Accessed November 10, 2015.

746 (2015y) Personal communication from FDA representative.

747 Baz M *et al* (2010) Effect of the neuraminidase mutation H274Y conferring resistance to oseltamivir on the replicative capacity and virulence of old and recent human influenza A(H1N1) viruses. *J Infect Dis* 201: 740-745

748 Kaminski MM *et al* (2013) Pandemic 2009 H1N1 influenza A virus carrying a Q136K mutation in the neuraminidase gene is resistant to zanamivir but exhibits reduced fitness in the guinea pig transmission model. *Journal of virology* 87: 1912-1915

749 Baz M *et al* (2006) Characterization of Multidrug-Resistant Influenza A/H3N2 Viruses Shed during 1 Year by an Immunocompromised Child. *Clin Infect Dis* 43: 1555-1561

750 Hai R *et al* (2013) Influenza A(H7N9) virus gains neuraminidase inhibitor resistance without loss of in vivo virulence or transmissibility. *Nat Commun* 4

751 (2015l) Interviews with influenza researchers.

GoF approaches that lead to evasion of therapeutics have the potential to benefit the development of new therapeutics in several ways:

- GoF approaches can be used to screen therapeutic candidates based on how readily various candidates acquire resistance and provide information about whether the emergence of resistance enhances the transmissibility or virulence of resistant viruses, an important aspect of safety testing.
- GoF approaches provide information about the potential field efficacy of the therapeutic and the mechanism of activity of the therapeutic, both of which are critical components of an Investigational New Drug application to the FDA.
- GoF approaches can provide insight into the therapeutic dosing regimens and combination therapies (e.g., cocktails of monoclonal antibodies) that are the least likely to permit evolution of resistance.

9.10.3.5.1 Therapeutic Development Benefit 1: Inform Development of Therapeutic Candidates

Given the high mutation rate of influenza viruses, viruses can readily acquire mutations to many therapeutics. Screening potential therapeutics based on how readily antiviral resistance emerges provides one mechanism for differentiating between therapeutic candidates based on their likely field efficacy. Prior to field deployment of a therapeutic, serially passaging viruses in the presence of therapeutic, a GoF approach, is uniquely capable of determining whether and how readily resistance arises. Furthermore, as resistance is expected to arise in human populations following deployment of the therapeutic, determining whether resistance enhances the infectivity, transmissibility, or virulence of a virus is an important aspect of safety testing of the therapeutic candidate.

9.10.3.5.2 Therapeutic Development Benefit 2: Facilitate Regulatory Approval of Therapeutic Candidates

The first step in the licensure process for new drugs involves submission of an Investigational New Drug (IND) application to the FDA's Center for Drug Evaluation and Research (CDER). CDER recommends that several types of nonclinical studies are conducted before starting Phase I clinical studies, including determination of the drug's mechanism of action, *in vitro* selection of resistant viruses to the investigational product, and the genotypic and phenotypic characterization of resistant viruses.[752] GoF approaches have the potential to support two aspects of an IND application for therapeutics in development: (1) determination of the mechanism of action of a therapeutic and (2) the *in vitro* selection of resistant viruses.

Determining the mechanism of action of a therapeutic

The FDA recommends that a drug's mechanism of action be "well-characterized" prior to the start of Phase I clinical trials and requests this information as a component of an IND application, the first step of the licensing process.[753] The influenza field is pursuing multiple strategies for developing new therapeutic candidates, including the deliberate design or selection of therapeutics targeting specific viral or host proteins and high-throughput screening of libraries of small molecules to identify compounds that reduce

[752] Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

[753] Ibid.

viral replication *in vitro*. In the former case, the drug target of the therapeutic candidate is known, while in the latter case, the therapeutic target is unknown. GoF approaches can be used to gain insight into the mechanism of activity of therapeutics that directly target virus proteins, thus benefitting the development of new drugs.

Passaging viruses in cells in the presence of a therapeutic is a classic method for generating viruses that can **evade the inhibitory action of the therapeutic**, thus constituting a GoF approach. Viruses are then sequenced to identify mutations that arose, and if multiple mutations are present, mutations are re-introduced into the parental strain individually and in combination to identify the minimal set of mutation(s) that are necessary and sufficient to confer antiviral resistance. Understanding which viral protein or proteins mutate in order for the virus to escape inhibition suggests those proteins are targeted by the therapeutic, and the site and phenotypic consequences of the mutations may provide insight into the mechanism of antiviral activity. Together, this information provides a foundation for follow-up structural, biochemical, and cell biological assays investigating the mechanism of antiviral activity. A major strength of this approach is that it can be applied to any type of therapeutic, including therapeutics with known targets (but unknown mechanisms of action) and therapeutics with unknown targets. However, elucidating the mechanisms of antiviral activity based on indirect observations about antiviral resistance can be challenging. For example, mutations may arise in proteins that are not directly targeted by the therapeutic, or the phenotypic consequences of mutations may be unclear.[754,755,756] Additionally, if the drug targets a host protein, this approach provides indirect information about its mechanism of activity.

Determining the genetic threshold for resistance development

Prior to the conduct of clinical trials and to support an IND application, the FDA recommends conducting *in vitro* studies for **selection of resistance to a therapeutic** in order to determine the genetic threshold for resistance development (i.e., how many mutations are needed to acquire resistance). Specifically, the FDA recommends passaging the virus in the presence of therapeutic, followed by sequencing of emergent resistant viruses and phenotypic characterization of resistant viruses.[757] This experiment constitutes a GoF approach.

9.10.3.5.3 Therapeutic Development Benefit 3: Inform Guidelines for Use of Therapeutics

The therapeutic regimen, including therapeutic dose and the use of combination therapies, may influence whether and how readily antiviral resistance arises. Given that influenza viruses readily acquire resistance to NAIs (i.e., upon acquisition of one or two mutations), influenza researchers cited a lack of knowledge about the potential utility of combination therapies as a critical gap in public health preparedness for influenza epidemics and pandemics.[758] In addition, understanding whether antiviral resistance arises more readily or differently in at-risk populations, such as obese or immunocompromised people, in either scenario can provide information that further refines therapeutic guidelines. GoF approaches can address each of these questions.

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754 Wensing AM *et al* (2014) 2014 Update of the drug resistance mutations in HIV-1. *Topics in antiviral medicine* 22: 642-650

755 Staschke KA *et al* (1995) Molecular basis for the resistance of influenza viruses to 4-guanidino-Neu5Ac2en. *Virology* 214: 642-646

756 Blick TJ *et al* (1998) The interaction of neuraminidase and hemagglutinin mutations in influenza virus in resistance to 4-guanidino-Neu5Ac2en. Ibid. 246: 95-103

757 Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

758 (2015l) Interviews with influenza researchers.

GoF approaches that lead to the development of viruses with **resistance to therapeutics in development** can be used to evaluate the relationship between emergence of resistance and therapeutic dosage or the administration of multiple therapeutics in combination. First, serial passaging of virus in cells or animals dosed with varying amounts of the therapeutic provides insight into the dose-dependence of the emergence of resistant viruses. Second, serial passaging of virus in cells or in animals in the presence of multiple therapeutics can be used to determine how readily resistance arises in response to combination versus single therapies. Finally, serial passaging of virus in mouse models for at-risk populations (e.g., immunocompromised mice or obese mice) provides additional information about the extent to which the likelihood of resistance or patterns of resistance mutations vary depending on host factors, which may inform therapeutic guidelines for specific at-risk populations.

9.10.3.6 Benefits and Limitations of GoF Approaches to the Development of Diagnostics

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[759]

9.10.3.7 Economic Benefits

GoF benefits to the development of therapeutics may have downstream economic benefits. Economic benefits were not explicitly evaluated in this report.

9.10.4 Identification of the Potential Benefits and Limitations of Alt-GoF Approaches That Provide Similar Potential Benefits to the GoF Approaches Being Examined

9.10.4.1 Benefits and Limitations of Alt-GoF Approaches to Scientific Knowledge

9.10.4.1.1 Scientific Knowledge Gap 1: What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?

Several GoF approaches enable the identification of mutations that confer antiviral resistance, including serial passaging and targeted genetic modification to introduce mutations associated with antiviral resistance. Conducting these approaches using attenuated reassortant strains in lieu of wild type strains represents one type of alternative approach. Specifically, because experiments in this phenotypic category focus on the influenza NA protein, reassortment strains containing the NA gene or the HA and NA genes from a seasonal strain of interest and the remaining six or seven genes from the lab-adapted, attenuated strain PR8 (7:1R or 6:2R strains) can be used. Influenza researchers felt that results about whether mutations do or do not confer antiviral resistance in the context of attenuated reassortant strains are generally reliable but cautioned that results may not be recapitulated in the context of the wild type strain.[760] Additionally, 6:2R and 7:1R strains cannot be used to discover or explore antiviral resistance that arises due to mutations in virus proteins other than the NA (or HA) genes.

Several alternative experimental approaches can also be used to identify mutations that lead to antiviral resistance. Comparative sequence analysis of wild type strains that are antiviral-resistant and antiviral-sensitive enables identification of mutations that are associated with antiviral resistance. However, because of the high genetic diversity among influenza viruses, identifying relevant mutations may be

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[759] New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

[760] (2015l) Interviews with influenza researchers.

difficult. One notable exception is comparative analysis of patient isolates over the course of antiviral treatment, which is more readily able to identify mutations associated with antiviral resistance due to the genetic similarity among patient isolates. However, the ability to opportunistically sample and analyze patient isolates is likely to be relatively rare. Furthermore, both studies provide associative information.

Forward genetic screens to identify mutations that restore antiviral sensitivity to antiviral-resistant strains (LoF) represents another alternative approach for discovering genetic traits linked to antiviral resistance. Because this approach involves screening mutants, it is less efficient than GoF approaches for the discovery of antiviral resistance traits, which rely on selection. Additionally, this approach is limited to the study of antiviral-resistant strains that have arisen in nature and cannot be used to proactively identify novel genetic traits that are associated with antiviral resistance. Targeted genetic modification of antiviral-resistant strains to introduce mutations expected to restore antiviral sensitivity can be used to demonstrate that a particular trait is necessary for antiviral resistance. Given that single mutations are typically sufficient to confer resistance to NAIs, targeted LoF and GoF approaches are equally capable of establishing a causal link between a particular genetic trait and antiviral-resistance. However, because use of the targeted LoF method relies on the existence of an antiviral-resistant strain carrying a particular resistance mutation of interest in nature, LoF is of limited utility for demonstrating that a resistance trait is conserved across multiple strain contexts than its GoF counterpart.

The use of *in vitro*, virus-free systems represents another alternative approach for the study of genetic traits underlying antiviral resistance. Several *in vitro*, virus free systems for the study of NAI resistance have been used, which rely on ectopic expression of the influenza NA gene in cell culture.[761,762] Using these systems, forward genetic screens can be used to discover novel mutations that confer resistance. Targeted mutagenesis of wild type NA genes can then be used to demonstrate that a particular mutation or set of mutations is necessary and sufficient to confer resistance, as well as to determine whether the phenotypic consequences of the mutation(s) are conserved across multiple genetic contexts. This approach can be successfully used to study mutations that confer resistance by altering the function of the NA protein. However, this approach cannot be used to uncover or to study mutations that confer resistance by altering the expression levels of the NA protein, as has been documented for the H275Y mutation (N1 numbering),[763] or mutations in other genes that give rise to resistance through epistatic effects. Additionally, given that antiviral-resistance is a continuum, results may not be recapitulated (or be clinically relevant) in the context of the full virus.

Finally, computational models have been used to predict mutations that disrupt binding between NAIs and the NA protein, which are expected to lead to antiviral resistance. While these models can be used to generate hypotheses about antiviral resistance mutations in any virus strain, all predictions must be experimentally confirmed through targeted mutagenesis, a GoF approach.

9.10.4.1.2 Scientific Knowledge Gap 2: What Selection Pressures Shape Whether and How Readily Antiviral-Resistant Strains Arise and Spread in Nature?

Several alternative approaches can be used to gain insight into selection pressures that shape the evolution and spread of antiviral resistance. Comparative analysis of the sequences and phenotypic characteristics of patient isolates over the course of antiviral treatment has potential to provide direct insight into the mechanisms driving emergence of antiviral resistance in people, including the identification of negatively

761 Nivitchanyong T *et al* (2011) Enhanced expression of secretable influenza virus neuraminidase in suspension mammalian cells by influenza virus nonstructural protein 1. *Journal of virological methods* 178: 44-51

762 Schmidt PM *et al* (2011) A Generic System for the Expression and Purification of Soluble and Stable Influenza Neuraminidase. *PLoS ONE* 6: e16284

763 Bloom JD *et al* (2010) Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. *Science (New York, NY)* 328: 1272-1275

selected traits. However, as these studies are typically conducted in immunocompromised patients due to their longer course of illness, results may not be representative of the general population. In addition, relative to animal passaging experiments (GoF), opportunities to conduct studies involving patients are likely to be relatively rare due to ethical considerations.

Comparative analysis of the phenotypic properties (e.g., fitness) of antiviral-resistant and antiviral-sensitive wild type strains can reveal genetic and phenotypic changes that are *associated* with the acquisition of antiviral resistance, which may provide insight into the viral properties that shape the evolution and spread of antiviral resistance in nature. However, the surveillance record is static and cannot provide insight into negatively selected traits. Moreover, current surveillance efforts, which largely involve consensus sequencing, are unlikely to capture the emergence of rare antiviral-resistant variants. For these reasons, comparative analysis of wild type viruses provides limited insight into the evolutionary mechanisms driving the evolution of antiviral resistance.

Other alternative approaches are not suitable for the study of evolutionary pressures that shape the emergence and spread of antiviral resistance. *In vitro*, virus free approaches cannot provide insight into how antiviral resistance affects other virus phenotypes, and current computational models cannot account for epistatic effects (e.g., how antiviral resistance affects fitness). The use of attenuated reassortant strains for GoF selection approaches, in lieu of wild type viruses, is of limited utility for studying the evolution of antiviral resistance because the fitness of attenuated strains is altered relative to the wild type strains.

9.10.4.2 Benefits and Limitations of Alt-GoF Approaches to Surveillance

Through influenza surveillance, public health professionals monitor the appearance and prevalence of NAI-resistant strains of seasonal influenza viruses circulating in global populations and of animal influenza viruses that have caused human infections. Resistance to NAIs can be assessed in two ways: through laboratory testing of NAI resistance and/or by inspecting sequences for the presence of mutations that are known to confer NAI resistance. As discussed in Section 9.10.3.2.2, the practice of using molecular markers is constrained by several sources of scientific uncertainty, namely whether known markers are conserved across multiple strain contexts and whether as-yet-unknown markers are capable of conferring resistance. This section first reviews the ability of alternative experimental approaches to address those scientific questions, then reviews the strengths and limitations of using phenotypic assays to assess the antiviral sensitivity of surveillance isolates (i.e., relative to molecular markers).

9.10.4.2.1 Utility and Limitations of Alt-GoF Approaches for Strengthening the Predictive Value of Molecular Markers for Antiviral Resistance

Alt-GoF approaches can be used to discover new mutations associated with antiviral resistance and to validate known markers for antiviral resistance in different genetic contexts, but they have significant limitations relative to GoF approaches. *In vitro*, virus-free systems can also be used to discover and validate new mutations that give rise to antiviral resistance by altering the function of the NA protein, but results may not be recapitulated in the context of the full virus. Targeted mutagenesis of antiviral-resistant strains to restore antiviral sensitivity (LoF) can establish a causal link between a particular trait and antiviral resistance, but the ability of this approach to demonstrate that particular markers are conserved across multiple strain contexts is limited relative to GoF approaches because it relies on the existence of antiviral resistant strains in nature. Comparing the sequences of wild type viruses or of patient isolates over the course of antiviral treatment enables the identification of mutations that are *associated* with antiviral resistance, which must be confirmed using targeted mutagenesis (GoF or LoF) to be useful for surveillance. In addition, these approaches are limited to the discovery of antiviral resistance mutations that have already arisen in nature. Computational models can be used to predict mutations that disrupt the interaction between an NAI compound and an antiviral, but predictions must be validated experimentally.

9.10.4.2.2 Strengths and Limitations of Using Phenotypic Assays to Characterize the Antiviral Sensitivity of Surveillance Isolates

The strength of phenotypic assays, relative to predictive approaches, is that phenotypic assays provide a direct readout of antiviral resistance. However, the practice of characterizing the antiviral sensitivity of surveillance isolates through phenotypic assays has several shortcomings. These shortcomings were discussed in detail in Section 9.6.3.2.1 and are briefly summarized here. First, the need for viral isolates limits the number of viruses that can be subjected to phenotypic characterization. Second, the composition of viral species present in the original clinical sample changes during isolation, as the most fit viral quasi-species outcompete others. This change is of particular concern for antiviral resistance testing because antiviral-resistant viruses are often less fit than antiviral-sensitive viruses, thus the presence of antiviral resistant strains in mixed infections can be obscured as a result of virus isolation. Finally, delays in shipping samples to WHOCCs for antiviral susceptibility testing, stemming from logistical, political, and/or regulatory factors, create a lag time between sample collection and phenotypic characterization.

9.10.4.3 Benefits and Limitations of Alt-GoF Approaches to Decision-Making in Public Health Policy

9.10.4.3.1 Benefits to Decision-Making Related to Seasonal Flu Strains

The CDC monitors the prevalence of antiviral resistance among circulating strains to inform their recommendations to clinicians for the use of influenza antivirals. A subset of strains collected through surveillance is subjected to phenotypic testing for antiviral resistance. Because phenotypic assays provide a direct readout of the antiviral sensitivity of a given strain, phenotypic testing is likely to remain a critical aspect of antiviral resistance monitoring in the future.

9.10.4.3.2 Benefits to Decision-Making Related to Pandemic Influenza

Antiviral resistance is one of the risk elements considered in pandemic risk assessments of animal influenza viruses, which inform downstream decision-making about investments in pre-pandemic preparedness initiatives (discussed in detail in Section 9.6.3.3.3). As described above (Section 9.10.3.3.2), the antiviral resistance element is important when coupled to other factors indicative of increased pandemic potential, such as a high number of human infections or enhanced transmissibility or virulence in ferrets. Importantly, when evaluating antiviral resistance stakeholders consider both phenotypic and genetic data, given the caveats associated with both types of data. Stakeholders emphasized that even following a rapid risk assessment based on sequence data, confirming the antiviral resistance phenotype through phenotypic testing is critical.

9.10.4.4 Benefits and Limitations of Alt-GoF Approaches to the Development of Vaccines

9.10.4.4.1 Vaccine Development Benefit: Targeted Mutagenesis to Remove Antiviral Resistance Markers from Vaccine Viruses

As discussed in Section 9.10.4.1.1, alt-GoF approaches are less efficient and effective than GoF approaches for the discovery of novel viral virulence traits. However, targeted mutagenesis of antiviral-resistant strains to introduce mutations expected to restore antiviral sensitivity (LoF), can be used to demonstrate that a particular amino acid or set of amino acids are *necessary* for antiviral resistance. As the ultimate goal is to restore antiviral sensitivity to vaccine strains harboring NAI-resistant NA genes, this approach is as suitable as its GoF counterpart for identifying molecular markers linked to antiviral resistance for this application.

9.10.4.5 Benefits and Limitations of Alt-GoF Approaches to the Development of Therapeutics

9.10.4.5.1 Therapeutic Development Benefit 1: Inform Development of Therapeutic Candidates

GoF approaches are used to screen therapeutic candidates based on how readily antiviral resistance emerges and also to determine whether emergence of resistance enhances the infectivity, transmissibility, or virulence of a virus, which is an important aspect of safety testing of the therapeutic candidate. No alternative approaches can provide this information prior to field deployment of a therapeutic.

9.10.4.5.2 Therapeutic Development Benefit 2: Facilitate Regulatory Approval of Therapeutic Candidates

Determining the mechanism of action of a therapeutic

The FDA recommends submitting information about the mechanism of action of a therapeutic as part of an IND application. This section reviews the ability of alt-GoF approaches to provide insight into the mechanism of action of a candidate therapeutics.

Therapeutic candidates that are identified through high-throughput screens may attenuate viral replication by directly targeting viral proteins or by indirectly targeting host proteins. For that reason, emergence of resistance studies, which investigate potential viral targets, are usually complemented by high-throughput RNAi screens targeting host proteins, to investigate potential host targets. Specifically, the fact that knockdown of a particular host protein impedes the drug's ability to inhibit viral replication suggests that that protein or that signaling pathway may be targeted by the therapeutic. Though an informative strategy for the study of therapeutics targeting host proteins, high-throughput RNAi screens provide minimal information about potential viral targets of therapeutics.

If the therapeutic target of a drug is known, analyzing the crystal structure of the viral target in complex with the antiviral compound (or mAb) can provide insight into the compound's mechanism of activity.[764,765] This approach is particularly useful for therapeutics that directly bind to and inhibit the activity of a viral protein. Though X-ray crystallography is appealing for its potential to provide direct information about the interaction between an antiviral and its target, inferring how that interaction affects a process in the viral life cycle may be difficult from such a static snapshot. Critically, because of the high level of effort required for X-ray crystallography, it is not a feasible approach for simply screening the potential viral targets of an unknown antiviral.

Photoaffinity cross-linking represents an alternative approach for identifying the binding site of a drug with a known target. In brief, this approach relies on the use of a "photoaffinity analogue" of the candidate therapeutic, which is synthesized to contain a photosensitive group (e.g., an azide) and a radioactive isotope (e.g., tritium, ^{3}H).[766] After treating the viral protein with the photoaffinity analog, the sample is irradiated with UV light, triggering the photosensitive group to form a covalent bond with the viral enzyme. Analytical techniques such as mass spectrometry can then be used to identify the labeled amino acid residues in order to determine the drug's binding site. This technique shares strengths and weaknesses with X-ray crystallography. Namely, photoaffinity cross-linking is useful for small molecule drugs that directly bind to and inhibit the activity of a viral protein and does not require prior knowledge

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764 Prabakaran P *et al* (2006) Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. *The Journal of biological chemistry* 281: 15829-15836

765 Ratia K *et al* (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences of the United States of America* 105: 16119-16124

766 Cohen KA *et al* (1991) Characterization of the binding site for nevirapine (BI-RG-587), a nonnucleoside inhibitor of human immunodeficiency virus type-1 reverse transcriptase. *The Journal of biological chemistry* 266: 14670-14674

of the location of the drug binding site.[767] However, inferring the mechanism of antiviral activity based on knowledge about the drug-virus protein interaction may be difficult.

Determining the genetic threshold for resistance development

Prior to the conduct of clinical trials and to support an IND application, the FDA recommends conducting *in vitro* studies for **selection of resistance to a therapeutic** in order to determine the genetic threshold for resistance development (i.e., how many mutations are needed to acquire resistance). The FDA guidance does not suggest any alternative approaches that could provide similar information. In fact, prior to deployment of the therapeutic and the emergence of resistant viruses in nature, no alternative approaches can provide this information.

9.10.4.5.3 Therapeutic Development Benefit 3: Inform Guidelines for Use of Therapeutics

GoF approaches can provide insight into the therapeutic dose that is least likely to lead to the emergence of resistance as well as whether combination therapies are less likely to lead to the emergence of resistance than single therapies. No alternative approaches are capable of providing similar information about the dose-dependence of resistance or whether combination therapies lead to resistance less readily than individual therapies.

9.10.5 Comparison and Analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

9.10.5.1 Benefits to Scientific Knowledge

9.10.5.1.1 Scientific Knowledge Gap 1: What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?

GoF approaches are uniquely capable of identifying mutations that are *necessary* and *sufficient* to confer antiviral resistance across multiple strain contexts, which provides a strong foundation for follow-up studies to elucidate the mechanisms underlying antiviral resistance. GoF approaches also represent the most efficient and effective approach for discovering novel mutations that confer antiviral resistance in any virus strain, as conducting experiments with wild type viruses allows for discovery of the full spectrum of mutations that may confer resistance, including mutations that alter the function or expression level of the NA gene as well as mutations in other virus proteins that cause resistance through epistatic effects. Attenuated reassortant strains may be used in lieu of wild type strains for many of these experiments, but results may not be recapitulated in the context of the wild type viruses, particularly if antiviral resistance arises through mechanisms other than changes to the function of the NA protein.

Alternative approaches can provide valuable insight into the study of antiviral resistance mechanisms but have limitations relative to GoF approaches. Discovering new genetic traits associated with antiviral resistance through comparative analysis of wild type sequences may be difficult. The comparison of patient isolates over the course of antiviral treatment is a notable exception, but opportunities for such studies are likely to be relatively rare. LoF approaches are relatively inefficient for the discovery of novel genetic traits associated with antiviral resistance but can be used to demonstrate that a particular mutation is necessary for antiviral resistance. Notably, the targeted LoF approach is often as capable of establishing a causal link between a particular mutation and antiviral resistance as the targeted GoF approach because NAI resistance is often conferred by single mutations; however, the ability of targeted LoF to demonstrate

[767] Hamouda AK *et al* (2014) Photoaffinity labeling of nicotinic receptors: diversity of drug binding sites! *Journal of molecular neuroscience : MN* 53: 480–486

that particular markers are conserved across strain contexts is limited by the number of antiviral resistant strains in nature. *In vitro* virus-free systems can be used to discover and validate mutations in the NA gene that give rise to resistance but are not suitable for the study of resistance mechanisms that involve alterations to gene expression levels or epistatic effects, and results may not be recapitulated in the context of the full virus. Computational models may be used to predict novel mutations that confer resistance by disrupting binding between the NAI molecule and the NA protein, but all predictions must be experimentally confirmed using GoF approaches.

9.10.5.1.2 Scientific Knowledge Gap 2 – What Selection Pressures Shape Whether and How Readily Antiviral-Resistant Strains Arise and Spread in Nature?

GoF approaches, namely serial passaging of viruses in animals in the presence of therapeutics, represent the most efficient and effective strategy for gaining in-depth insight into the viral and host selection pressures that shape the emergence and spread of antiviral resistance. Notably, attenuated reassortant strains cannot be used for these studies because the phenotypic properties that are likely to shape the likelihood that antiviral resistant strains will spread and persist in human populations, such as fitness, are altered in these strains. While gaining direct insight into the behavior of the virus in humans through human challenge studies (GoF) is valuable, these studies are rare due to ethical considerations. Comparative analysis of patient isolates over the course of antiviral treatment can also provide in-depth insight into the evolution of antiviral resistance in people, but studies are typically conducted in immunocompromised patients and thus may not translate to healthy populations. Comparative analysis of wild type isolates provides limited mechanistic insight into the viral or host factors that shape evolution of antiviral resistance. Finally, neither virus-free approaches nor *in silico* approaches can be used to study the interplay between antiviral resistance and other virus phenotypes.

9.10.5.2 Benefits to Surveillance

Through influenza surveillance, public health professionals monitor the appearance and prevalence of NAI-resistant strains of seasonal influenza viruses circulating in global populations and of animal influenza viruses that have caused human infections. Resistance to NAIs can be assessed in two ways: through laboratory testing of NAI resistance and/or by inspecting sequences for the presence of mutations that are known to confer NAI resistance. GoF approaches have the potential to benefit surveillance for antiviral resistant strains by strengthening the predictive value of molecular markers for antiviral resistance.

9.10.5.2.1 Benefits of GoF Approaches Relative to Alt-GoF Approaches for Strengthening the Predictive Value of Molecular Markers for Antiviral Resistance

GoF approaches represent the most efficient and effective methods for discovering novel mutations associated with antiviral resistance and are uniquely capable of demonstrating that a particular mutation is necessary and sufficient to confer antiviral resistance across multiple strain contexts. Alternative approaches have significant limitations for the discovery of new markers and for the validation of known markers. Taken together, GoF approaches provide unique benefits for strengthening the predictive value of molecular markers for antiviral resistance, thereby improving their utility for interpreting surveillance data.

9.10.5.2.2 Benefits of Using Molecular Markers (GoF) Versus Phenotypic Assays (Alt-GoF) to Characterize the Antiviral Sensitivity of Surveillance Isolates

Both phenotypic assays and inspection of sequences for molecular markers of antiviral resistance have strengths and limitations. Phenotypic assays provide direct information about the degree of antiviral

resistance of a particular strain, but results are delayed relative to sample collection and the properties of viral isolates may not reflect the properties of viral quasispecies present in the original clinical sample. For these reasons, researchers and government officials involved in influenza surveillance value the ability to corroborate phenotypic assay data with sequence-based predictions based on molecular markers of antiviral resistance, particularly when clinical samples can be directly sequenced. Of note, NAI resistance markers that have been shown to be conserved across multiple strain contexts and are currently incorporated into the practice of analyzing surveillance data.[768] Thus, the benefits of GoF research about molecular markers for antiviral resistance to the practice of surveillance can be realized immediately. As sequencing becomes more common at NICs and other diagnostic laboratories and the number of known, validated markers for NAI resistance rises, the molecular marker approach will take on relatively greater importance. Ultimately, due to the rapidity of sequence-based analysis relative to phenotypic assays, the use of molecular markers may increase capacity to monitor for antiviral resistance.

Notably, most genetic surveillance data is generated by sequencing of viral isolates at WHOCCs, though the number of NICs and other diagnostic labs with sequencing capabilities is rising (though most of these labs carry out sequencing on viral isolate samples). Full realization of the benefits that can be derived from the use of molecular marker data will require an expansion of sequencing capabilities at diagnostic laboratories that comprise the "base" of the influenza surveillance system as well as increasing the number of clinical samples that are directly sequenced. Both capabilities have increased over the past decade and are expected to continue to increase.[769]

9.10.5.3 Benefits to Decision-Making in Public Health Policy

As described above, GoF approaches have potential to benefit surveillance for antiviral resistant strains, which informs downstream decision-making related to public health practice and policy. Namely, data on the prevalence of antiviral-resistant seasonal strains informs therapeutic recommendations developed by the CDC, and antiviral resistance is one of the risk elements considered in a pandemic risk assessment of an animal influenza virus.

Currently, a subset of the seasonal influenza viruses collected by WHOCCs are sent to CDC for antiviral susceptibility testing.[770] As discussed above, the use of molecular markers (GoF) has potential to strengthen the robustness of antiviral resistance data, by corroborating phenotypic assay data, and to increase the number of strains that can be phenotypically characterized. This expansion will provide a stronger foundation for antiviral treatment recommendations and may enhance detection of rare antiviral-resistant variants to increase awareness. However, given the inherent uncertainty of sequence-based predictions, researchers and governmental officials involved in the analysis of surveillance data emphasized that predictions should be validated through antiviral resistance assays whenever possible.

For pandemic risk assessments, the observation of antiviral resistance does not increase risk a priori but rather is important when coupled to other factors indicative of increased pandemic potential, such as a high number of human infections or enhanced transmissibility or virulence in ferrets. In this case, the observation of antiviral resistance may trigger USG representatives to initiate the process of applying for Emergency Use Authorization (EUA) from the FDA for antivirals in development, to ensure that effective antivirals will be available if the strain under evaluation spreads to cause a pandemic. Importantly, when evaluating antiviral resistance, stakeholders value the ability to corroborate phenotypic data with analysis of genetic data, given the caveats associated with phenotypic assays. Additionally, the ability to conduct a rapid risk assessment based on sequence data is valuable when strains first emerge and sequences are

[768] (2015f) Interviews with influenza researchers and U.S. government representatives involved in influenza surveillance.
[769] (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.
[770] Centers for Disease Control and Prevention. Antiviral Drug Resistance among Influenza Viruses. http://www.cdc.gov/flu/professionals/antivirals/antiviral-drug-resistance.htm. Last Accessed: November 4, 2015

published prior to the receipt of viral isolates. Early decision-making can provide a several week head start on the EUA process. As EUAs may be issued within days following a request if the FDA has worked with government partners on a "pre-EUA" package, this head start could significantly impact the timing of availability of antivirals in the event of a pandemic.[771,772,773]

9.10.5.4 Benefits to the Development of Vaccines

Antiviral resistance markers can be mutated out of vaccine viruses to increase the safety of the vaccine production process. Although GoF approaches represent the most efficient and effective way to discover novel markers for antiviral resistance, either targeted GoF or LoF approaches can be used to establish a causal link between a particular genetic marker and antiviral resistance, which is needed for translation of this information to the vaccine production process. As the ultimate goal is to restore antiviral sensitivity to vaccine strains harboring NAI-resistant NA genes, either GoF or alt-GoF approaches are equally suitable for identifying molecular markers linked to antiviral resistance for this purpose.

9.10.5.5 Benefits to the Development of Therapeutics

9.10.5.5.1 Therapeutic Development Benefit 1: Inform Development of Therapeutic Candidates

GoF approaches are uniquely capable of screening potential therapeutic candidates based on how readily antiviral resistance emerges as well as determining whether the emergence of resistance enhances the infectivity, transmissibility, or virulence of a virus, an important aspect of safety testing of the therapeutic candidate.

9.10.5.5.2 Therapeutic Development Benefit 2: Facilitate Regulatory Approval of Therapeutic Candidates

The FDA recommends that an IND application include information about the mechanism of action of the proposed therapeutic as well as selection for resistance studies to demonstrate the genetic threshold for resistance to the therapeutic.

Serial passaging of a virus in the presence of therapeutic to discover mutations that confer resistance, a GoF approach, is uniquely capable of identifying the viral target of a novel therapeutic with an unknown mechanism of action. Given that researchers are undertaking unbiased screens to identify candidate therapeutics that inhibit viral replication, this represents a valuable benefit for the development of new influenza therapeutics. For therapeutics with known viral targets, this information about resistance mutations can provide foundational information to guide follow-up structural, cell biological, and biochemical studies investigating the mechanism of action of the therapeutic. Although crystallography and photoaffinity cross-linking can also provide insight into the antiviral mechanisms of therapeutics that directly bind to and inhibit virus proteins, inferring mechanistic information based on static information about the virus-antiviral complex may be difficult. In these cases, knowledge about mutations that confer resistance, generated through GoF approaches, provides an additional source of information that can be used to generate testable hypotheses about mechanism of antiviral activity. Finally, the identification of host factors that are required for antiviral activity is a critical aspect of examining therapeutics with unknown targets. Though solely using host-focused approaches to elucidate the antiviral mechanism of a

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771 Administration FaD. Guidance - Emergency Use Authorization of Medical Products. http://www.fda.gov/RegulatoryInformation/Guidances/ucm125127.htm. Last Update Accessed November 10, 2015.

772 FDA. Emergency Use Authorization of Medical Products. http://www.fda.gov/RegulatoryInformation/Guidances/ucm125127.htm. Last Update October 22, 2014. Accessed November 28, 2015.

773 (2015y) Personal communication from FDA representative.

therapeutic that targets the virus would be difficult, this information complements GoF approaches to strengthen the evidence base for the drug's mechanism of action.

GoF approaches are uniquely capable of determining the genetic threshold for resistance to a new therapeutic, prior to field deployment of that therapeutic.

9.10.5.5.3 Therapeutic Development Benefit 3: Inform Guidelines for Use of Therapeutics

GoF approaches that lead to the generation of viruses that are resistant to therapeutics in development are uniquely capable of determining the therapeutic dose that is least likely to lead to the acquisition of antiviral resistance as well as determining whether combination therapies better prevent the emergence of resistant viruses than individual therapies. Both types of information benefit the development of therapeutic strategies that will be effective for a longer period of time in the field.

9.11 Influenza Viruses: Benefits of GoF Research Involving Reassortment

9.11.1 Summary

This section describes the benefits of GoF studies that investigate the reassortment potential between two viruses, which may lead to one or more of the phenotypic changes detailed in the NSABB Framework. Such GoF studies were found to generate scientific knowledge, to inform surveillance of circulating animal influenza viruses, which has downstream impacts on decision-making about USG investments in pandemic preparedness initiatives such as pre-pandemic vaccine development, and to inform community-level interventions aimed at preventing the emergence of novel reassortant viruses in human populations. Alt-GoF approaches that may generate similar benefits were also identified and analyzed. At present, GoF studies that involve reassortment have unique benefits to scientific knowledge and public health practice, but realization of benefits to surveillance is subject to significant improvements to surveillance capabilities for reassortant viruses. Chapter 9.11 provides an overview of these benefits, including basic background and Supporting Information; a fully referenced and more thorough discussion of these benefits can be found in Appendix IV Section 15.8.

9.11.1.1 Benefits of GoF Studies That Involve Reassortment to Scientific Knowledge

- GoF approaches are:
  - Uniquely capable of proactively assessing the potential for any two influenza viruses to reassort and of comprehensively evaluating the viability of various gene combinations. However, the outcomes of forced reassortment events in the laboratory may not reflect what is possible or likely to occur in nature, and results from animal models may not translate to human disease.

- Alt-GoF approaches are:
  - Uniquely capable of studying co-infection and reassortment events in nature, but provide limited mechanistic insight due to the complexities of the interactions between the viruses, the host, and environmental factors that influence reassortment outcomes.

9.11.1.2 Benefits of GoF Studies That Involve Reassortment to Surveillance

- GoF approaches:
  - May inform rapid assessment of the risks posed by circulating reassortant viruses detected through surveillance, although the value of this benefit is limited by the fact that laboratory

results may not translate to viruses observed in nature. In addition, full realization of this benefit will require significant improvements to surveillance capabilities for reassortant viruses.

- Alt-GoF approaches:
  - May inform assessment of the risks posed by circulating reassortant viruses detected through surveillance, although this information is subject to the availability of surveillance isolates and will be delayed relative to the application of GoF data. In addition, full realization of this benefit will require significant improvements to surveillance capabilities for reassortant viruses.

9.11.1.3 Benefits of GoF Studies That Involve Reassortment to Public Health Practice and Policy

- GoF approaches:
  - GoF approaches may help to prioritize community-level interventions that aim to limit cross-species interactions that would provide opportunities for co-infection between human seasonal viruses and animal influenza viruses that have not yet infected humans. This benefit arises from assessment of the potential and consequences of reassortment events, which is one aspect of the risk posed by reassortment to human populations.
  - GoF data may inform pandemic risk assessments of animal influenza viruses, which guide downstream decision-making about pre-pandemic vaccine development and other pandemic preparedness initiatives. GoF data are of low importance relative to other factors considered in the risk assessment but can play an important role in rapid risk assessments when novel viruses first emerge in human populations.
- Alt-GoF approaches:
  - Alt-GoF approaches may help to prioritize community-level interventions that aim to limit cross-species interactions that would provide opportunities for co-infection between human seasonal viruses and animal influenza viruses that have not yet infected humans. This benefit arises from new insights into the ecological factors that shape the likelihood of reassortment events occurring in nature, which is another aspect of the risk posed by reassortment to human populations. Thus, this data complements that generated by GoF approaches to refine prioritization of "prevention" activities.
  - Alternative factors considered in the risk assessment, in particular epidemiological and virologic factors, have a higher weight than the genomic variation risk element (informed by GoF). However, these data may be scant or delayed relative to sequence data when novel viruses first emerge in human populations.

9.11.2 Overview of GoF Research That Involves Reassortment

This assessment describes the benefits of GoF experimental approaches that aim to assess the genetic compatibility and fitness of viruses following reassortment. While the phenotypic consequences of reassortment events between two viruses cannot be predicted with certainty, reassortant strains may exhibit enhanced fitness, pathogenicity, and/or transmissibility relative to one or both parental strains. (Notably, reassortant strains may also display *reduced* fitness, pathogenicity, and/or transmissibility relative to parental viruses.) In this section, we provide an overview of GoF approaches that can be used to assess the reassortment potential between two viruses and describe the scientific outcomes of each approach.

9.11.2.1 Targeted Reassortment by Combining Viral Gene Segments from Two or More Viruses to Generate Viable Reassortant Viruses

Targeted reassortment of virus gene segments from two or more wild type virus isolates followed by characterization of fitness in cell culture or in representative animal models is used to assess genetic compatibility. This approach is in part performed to evaluate the genetic compatibility and viability of a *single* reassortant virus, which can inform the understanding of the mechanisms underlying genetic compatibility between virus gene segments across virus strains and subtypes. For example, a reassortant virus compromised of virus gene segments sharing homology to the 1918 H1N1 pandemic virus from eight different wild type avian isolates was generated to demonstrate that some 1918-like avian viruses circulating in nature (which reassort frequently) are genetically compatible.[774]

9.11.2.2 Forward Genetic Screen to Identify Viable Reassortant Viruses

Forward genetic screens involve the generation of a panel of clonal recombinant viruses by comprehensive reassortment of parental gene segments from two viruses (i.e., all or many possible gene combinations), followed by characterization of the fitness of reassortants in appropriate mammalian model systems. Follow-up studies may be performed to evaluate pathogenicity, infectivity, and/or transmissibility of viable reassortants. This approach is performed to evaluate viability and genetic compatibility of reassortant viruses, which provides a foundation for studies investigating mechanisms governing reassortment and informs the potential for reassortant viruses to emerge in nature and the potential public health consequences of such an emergence event.

9.11.2.3 Non-Targeted Reassortment Using Reverse Genetics to Select for Viable Reassortant Viruses

In this approach, reassortants are generated using reverse genetics to mix viral gene segments of two wild type viruses (i.e., mix up to 16 gene segments in total) in the context of cell culture or animal models. Use of cell culture model systems involves the transient transfection of viral gene segments, while the *in vivo* method involves the inoculation of ferrets with transiently transfected cells, followed by viral reassortment *in vivo*. Both approaches are followed by limited passaging to select for viable reassortants. Clonal isolates that emerge are then genotyped to identify their gene composition. This approach provides insight into viable gene reassortment combinations as well as the relative fitness of reassortants under selection pressures, which informs the potential and likelihood of reassortment emergence in nature.

9.11.2.4 Co-Infection to Select for Viable Reassortant Viruses

In this approach, cultured cells or representative animal models are co-infected with two different wild type viruses, followed by genotyping of clonal isolates that emerge during co-infection. This approach determines the viability of various gene reassortment combinations *and* the relative fitness of reassortants under selection pressures, which can inform the potential and likelihood of emergence in nature.

9.11.3 Identification of the Potential Benefits and Limitations of GoF Approaches

In this section, the potential benefits of GoF experiments that investigate the reassortment potential between two viruses are discussed, in each benefit category listed in the NSABB Framework.

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[774] Watanabe T *et al* (2014a) Circulating avian influenza viruses closely related to the 1918 virus have pandemic potential. *Cell Host Microbe* 15: 692-705

9.11.3.1 Scientific Knowledge

9.11.3.1.1 Background – Critical Gaps in Scientific Knowledge About Reassortment

Here, the ability of GoF approaches to address a key outstanding question related to the reassortment of influenza viruses in humans and other host species is evaluated:

- What is the potential for reassortment between two influenza virus strains?
  - Are two influenza viruses genetically compatible?
  - What is the relative fitness of reassortants that may affect the likelihood of their emergence under selection in a host?
  - How do selection pressures influence reassortment?

Reassortment involves the exchange of one or more complete virus gene segments between two different viruses during the co-infection of a single cell, which can result in viruses that display enhanced fitness, immune evasion and antigen escape, and resistance to antivirals.[775] Considerable gaps in knowledge remain about the biology of reassortment, including whether reassortment between two viruses will occur and will lead to the generation of viruses with enhanced fitness, pathogenicity, and/or transmissibility. GoF approaches can provide insight into these questions.

9.11.3.1.2 Benefits and Limitations of GoF Approaches That Study Reassortment

Several GoF approaches can lead to the generation of reassortant viruses:

- Targeted reassortment to generate a virus comprised of gene segments from two or more wild type isolates.
- Forward genetic screens involving comprehensive reassortment to generate a panel of clonal viral isolates followed by assessment of fitness in cell culture or representative animal models.
- Non-targeted reassortment involving gene segments from two different viruses to generate a mixed population of reassortant viruses, followed by selection for compatible virus genotypes in cell culture or representative animal models, and
- Co-infection of cell culture or representative animal models with two different viruses to select for compatible virus genotypes.

Collectively, these approaches definitively demonstrate whether reassortment can occur between wild type viruses and enable the identification of reassortment gene combinations that permit replication in *in vitro* or *in vivo* model systems. This provides insight into the genetic compatibility of virus gene segments. For the targeted reassortment approach, viral gene segments are selected based on a property of interest (e.g., homology to a human pandemic virus) to answer a specific question about the genetic compatibility between two or more viruses, which differs from the other GoF approaches that more broadly query the range of reassortment combinations that are possible between two viruses.

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[775] Steel J, Lowen AC (2014) Influenza A virus reassortment. *Current topics in microbiology and immunology* 385: 377-401

Because forward genetic screens individually test every possible gene combination between two viruses, this GoF approach can assess the viability and fitness of *each* viral clone that may otherwise be missed with selection based approaches (below) in which more fit clones outcompete. However, the outcomes associated with forward genetic screens are independent of the selection pressures that shape reassortment potential and viral population diversity, and therefore may not fully represent the likelihood of reassortants emerging.

The use of non-targeted reassortment by transfection of cell culture model systems with gene segments from two separate viruses to select and identify emergent reassortants presents several different advantages. First, this approach provides insight into how host pressures and competition among reassortants shapes outcomes. Second, the ability to selectively remove a single gene segment that may otherwise outcompete or skew virus populations enables assessment of the compatibility of many gene segment combinations, relative to the co-infection approach.

Similar to the non-targeted reassortment approach, the co-infection approach provides insight into how the host pressure and competition impact selection. A major benefit of this approach is that it mimics the natural scenario under which reassortment can occur. However, in the event that two viruses of interest display different tissue and cell tropism or significant disparity in fitness or infectivity, this approach permits study of a limited number of reassortment combinations.

For all three approaches, the use of *in vivo* animal models for reassortment studies provides more relevant information due to the complexity of host selection pressures relative to cell culture models. All GoF approaches described here depend on whether the mechanisms and selection pressures underlying fitness and reassortment in cell culture or animal models are representative of those in humans and whether the genetic compatibility observed for the select number of strains analyzed is generalizable in other virus contexts. Moreover, the use of the methods above may not capture the dynamics of co-infection and reassortment in nature, limiting the relevance of results.

9.11.3.2 Surveillance

9.11.3.2.1 Surveillance for Reassortant Viruses – Current Process and Limitations

The importance of reassortment in influenza virus biology is highlighted by its role in the emergence of human pandemic viruses with minimal population immunity – all four of the influenza pandemics that have occurred over the past century were likely caused by strains that arose through reassortment between influenza viruses, although the role of reassortment in the emergence of the 1918 pandemic virus is controversial.[776,777,778,779,780] While the emergence of reassortant viruses cannot yet be predicted, surveillance for reassortant viruses to assess their occurrence and prevalence in nature is of interest for pandemic preparedness, and as such is one of the factors considered in pandemic risk assessments (discussed further below). Determining whether a reassortant virus poses an increased risk to human populations relative to its parental viruses poses a major challenge.

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[776] Morens DM, Fauci AS (2007) The 1918 influenza pandemic: insights for the 21st century. *The Journal of infectious diseases* 195: 1018-1028

[777] Belshe RB (2005) The origins of pandemic influenza--lessons from the 1918 virus. *The New England journal of medicine* 353: 2209-2211

[778] Worobey M *et al* (2014) Genesis and pathogenesis of the 1918 pandemic H1N1 influenza A virus. *Proc Natl Acad Sci U S A* 111: 8107-8112

[779] Steel J, Lowen AC (2014) Influenza A virus reassortment. *Current topics in microbiology and immunology* 385: 377-401

[780] Smith GJ *et al* (2009b) Dating the emergence of pandemic influenza viruses. *Proc Natl Acad Sci U S A* 106: 11709-11712

Analysis of the phenotypic properties of reassortant viruses in a laboratory setting, in particular fitness, pathogenicity, and transmissibility, provides insight into the properties associated with viable reassortants and can call attention to particular reassortant viruses that display phenotypic properties of concern. This information can inform evaluation of the risk posed by particular reassortant viruses detected in nature.

9.11.3.2.2 Benefits and Limitations of GoF Approaches to Surveillance for Reassortant Viruses

GoF approaches that proactively determine the reassortment potential between two viruses and phenotypic properties of reassortant viruses represent an efficient method for generating a breadth of information that can inform rapid analysis of surveillance data. However, whether laboratory results translate to the field strains of interest in nature is uncertain, given differences in the genetic sequences of the laboratory and field strains and the inherent artificiality of studies conducted in model systems in a laboratory setting.

9.11.3.3 Decision-Making in Public Health Practice and Policy

GoF reassortment studies have potential to benefit two aspects of public health practice and policy. First, the results of reassortment studies may stimulate risk mitigation activities to limit the potential for risky co-infections to occur in nature in human and/or animal hosts (i.e., those co-infections that could give rise to reassortant viruses with risky properties). Second, reassortment studies may inform pandemic risk assessments of circulating animal influenza viruses, which guide downstream decision-making about pre-pandemic vaccine development and other pandemic preparedness initiatives.

9.11.3.3.1 GoF Benefits to Risk Mitigation Activities That Aim to Prevent the Emergence of Reassortant Viruses in Nature

Reassortant viruses arise in nature during co-infection of a host with two different viruses. Limiting the interaction between two different species can mitigate the risk of co-infection of either host with an adapted and an "exotic" strain (e.g., co-infection of a human with seasonal H1N1 and avian H5N1), which could give rise to a reassortant strain comprised of viral gene segments from both strains.[781] GoF approaches that proactively study the reassortment potential between two virus strains adapted for growth in different species provides insight into reassortants that are viable and that display phenotypic properties of concern. This information can help to prioritize risk communication about measures to mitigate the chance of co-infections.[782] For example, hunters would be encouraged to wear personal protective equipment while gutting birds in areas where avian viruses that are capable of reassorting with human seasonal viruses are circulating in game bird populations.[783] Furthermore, data from GoF reassortment studies provides an evidence base for messaging that may increase "buy-in" among the target population. The results of GoF reassortment studies may also inform biosecurity practices at farms, with respect to interactions between farm workers and animals, interactions between different species of animals (e.g., poultry and swine at mixed-species farms), and interactions between agricultural animals and wildlife.

Environmental conditions that provide opportunities for co-infections with a human seasonal virus and an animal flu virus that has already caused human infections are of high concern regardless of results from laboratory reassortment studies.[784] Thus, GoF studies that investigate the reassortment potential between

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781 (2015q) Interviews with researchers at the National Wildlife Health Center (United States Geological Survey, Department of the Interior).

782 (2015l) Interviews with influenza researchers.

783 (2015q) Interviews with researchers at the National Wildlife Health Center (United States Geological Survey, Department of the Interior).

784 Zhu Y *et al* (2013a) Human co-infection with novel avian influenza A H7N9 and influenza A H3N2 viruses in Jiangsu province, China. *Lancet* 381: 2134

human seasonal viruses and animal viruses that have not yet caused human infections are likely to have a larger impact on public health practice (e.g., HPAI H5N2 and human seasonal viruses).

9.11.3.3.2 GoF Benefits to Pandemic Risk Assessments and Downstream Decision-Making for Pandemic Preparedness

Pandemic risk assessments of circulating animal influenza viruses inform decision-making about how to invest in public health preparedness activities for influenza pandemics, particularly development of pre-pandemic vaccines. The genomic variation risk element of the Influenza Risk Assessment Tool (IRAT) used by the USG for pandemic risk assessments, described in detail in Section 9.6.3.3.2, includes consideration of reassortment. Specifically, reassortment between different lineages or sub-types of viruses raises the risk score for this element. GoF approaches that provide insight into the properties of reassortant viruses, in particular their fitness, transmissibility, and virulence, could be used to refine the scores associated with this risk element. In this way, GoF approaches may benefit downstream decision-making in public health policy.

In general, the genomic variation risk element is of low to intermediate importance relative to other factors considered in the risk assessment, such as the number of human infections and the phenotypic properties of the virus. Notably, corroboration of phenotypic data adds value to the assessment by increasing certainty in downstream decisions. Furthermore, as discussed in detail in Section 9.6.3.3.3, the genomic variation risk element may play a relatively more important role in the assessment when a novel virus first emerges in human populations, if sequences are published prior to the shipping of viral isolates to the US. The ability to evaluate risk based on genetic sequence data enables a rapid risk assessment, which may trigger the decision to develop a CVV, providing a head start on vaccine production that would be valuable in the event of a pandemic.

9.11.3.4 Vaccines, Therapeutics, and Diagnostics

GoF-derived information about the reassortment potential of two different viruses is not relevant for the development of vaccines or therapeutics.

As existing influenza diagnostics are not equipped to rapidly screen and detect reassortants, information about reassortants with phenotypic properties of concern could, in principle, guide development of diagnostics to detect those reassortants. However, GoF approaches do not provide insight into the likelihood that reassortment will occur in nature, which is a function of complex ecological factors that govern the likelihood of co-infections. The likelihood of reassortment is also a critical factor for the design of targeted diagnostics for reassortant viruses (i.e., there is no need to design diagnostics for rare reassortant events). For this reason, GoF approaches are unlikely to trigger the development of new diagnostics independently of the observation of co-infection or reassortment events occurring in nature.

9.11.3.5 Economic Benefits

No economic benefits of GoF reassortment studies were identified.

9.11.4 Identification of the Potential Benefits and Limitations of Alt-GoF Approaches That Provide Similar Potential Benefits to the GoF Approaches Being Examined

9.11.4.1 Scientific Knowledge

A select number of alt-GoF approaches can be used to analyze the reassortment potential of two different viruses. Analyzing the sequences of human and animal surveillance isolates to detect reassortment events

can provide insight into the occurrence and prevalence of reassortment in nature. This approach includes sequence inspection for several different types of reassortment events, involving:

- Two different human seasonal virus sub-types (e.g., H1N1 and H3N2),
- Human or animal virus strains within the same sub-type (e.g., different clades of H3N2),
- Human and animal viruses (e.g., human seasonal H3N2 and swine-origin H1N1), and
- Two different animal virus sub-types (e.g., H9N2 and H7Nx).

Analysis of both animal and human isolates provides information that is applicable to a broad number of strains, and the analysis of human isolates provides information about reassortment potential that is directly relevant to human populations. However, this approach is significantly limited by the quality and availability of existing genetic surveillance data. In addition, this analysis is limited to the study of reassortant viruses that have evolved (and have been subsequently detected) in nature.

A second type of alt-GoF approach involves the analysis of viral isolates from humans or animals that have been co-infected with two influenza viruses. This approach can determine whether reassortment has occurred and also may provide insight into the genetic compatibility of various gene combinations, as well as host selection pressures that shape the outcome of reassortment events. That analysis of human and animal isolates provides information that is directly relevant to reassortment potential in nature is a strength of this method. However, this approach is also subject to significant limitations. Although co-infection events occur, these events are captured on an ad hoc basis, thus opportunities for such studies are likely to be relatively rare. Moreover, unknowns in the route of infection, the level and time of exposure, and diversity in the host response due to existing natural or induced immunity limits the ability of this approach to reliably assess genetic compatibility of reassortant viruses.

The use of replication incompetent viruses provides another alternative method for the analysis of genetic compatibility between gene segments from two influenza viruses. In these model systems, viral replication can be assessed in cell culture lines that are engineered to stably express an essential viral protein that is missing from the "replication-incompetent" virus strains used for infection. The result is a virus that is biologically constrained to replication in that cell line. Several replication incompetent model systems have been made, and these systems have been used to assess the of genetic compatibility of specific virus gene segments by targeted.[785,786,787] However, this system has not yet been used to broadly assess the reassortment potential between two viruses (i.e., reassortant viruses that emerge following transfection of cells with all eight gene segments from both viruses, which mimics a co-infection event). One major drawback is that this approach does not capture the complex selection pressures observed *in vivo*. Additionally, results may not translate to reassortment in humans, and findings may not be generalizable to other virus contexts.

A final alt-GoF approach utilizes *in vitro* virus-free methods to investigate genetic compatibility of viral gene segments in isolation. In particular, forward genetic screens can be used to identify novel gene segment combinations or reassortment events that contribute to a phenotype underlying viral fitness and infectivity, such as polymerase activity. Though the simplicity and relatively high-throughput nature of these methods renders them appealing as a screening approach for the evaluation of genetic compatibility between two viruses, these approaches are inherently limited to the characterization of phenotypes

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[785] Ozawa M *et al* (2011) Replication-incompetent influenza A viruses that stably express a foreign gene. *The Journal of general virology* 92: 2879-2888

[786] Martinez-Sobrido L *et al* (2010) Hemagglutinin-Pseudotyped Green Fluorescent Protein-Expressing Influenza Viruses for the Detection of Influenza Virus Neutralizing Antibodies. *Journal of virology* 84: 2157-2163

[787] Baker SF *et al* (2014) Influenza A and B virus intertypic reassortment through compatible viral packaging signals. *Journal of virology* 88: 10778-10791

previously identified in other experiments. In addition, results may not be recapitulated in the context of the full virus or *in vivo*.

9.11.4.2 Surveillance

Analysis of the phenotypic properties of reassortant viruses in a laboratory setting, in particular fitness, pathogenicity, and transmissibility, provides insight into the properties associated with viable reassortants and can call attention to particular reassortant viruses that display phenotypic properties of concern. This information can inform evaluation of the risk posed by particular reassortant viruses detected in nature.

Characterization of field viruses, an alt-GoF approach, provides direct insight into the phenotypic properties of reassortant viruses of interest. However, this approach is reactive and depends on the availability of viral isolates or the publication of a high-quality, complete genome sequence for synthetic reconstruction of the virus. Additionally, this approach provides limited mechanistic insight into the relative fitness of reassortant and parental viruses, due to the high genetic diversity among circulating influenza viruses. Finally, whether results gleaned from studies in laboratory animals translate to human disease is uncertain.

9.11.4.3 Decision-Making in Public Health Practice and Policy

9.11.4.3.1 Alt-GoF Benefits to Risk Mitigation Activities That Aim to Prevent the Emergence of Reassortant Viruses in Nature

Reassortant viruses arise in nature during co-infection of a host with two different viruses. Limiting the interaction between two different species can mitigate the risk of co-infection of either host with an adapted and an "exotic" strain (e.g., co-infection of a human with seasonal H1N1 and avian H7N9), which could give rise to a reassortant strain.[788] Understanding whether reassortment between two viruses has potential to generate viruses with phenotypic properties of concern (e.g., enhanced transmissibility, virulence, etc.) can inform prioritization of community-level interventions that aim to limit opportunities for "risky" co-infection events. Because alternative experimental approaches are reactive, limited to study reassortment events that have already occurred in nature, these approaches have limited ability to inform such proactive "prevention" initiatives.

However, the risk posed to human populations by reassortment events also depends on the likelihood that co-infections and subsequent reassortment occurs. The likelihood of reassortment in nature depends on complex ecological factors such as the distribution of viruses within and among reservoir species, which are poorly understood. These factors can be studied using alternative approaches such as characterizing the prevalence and distribution of influenza viruses circulating within and between animal reservoir species. This information can provide insight into the factors that drive reassortment events in nature, which will help to refine risk communication and community-level intervention efforts that aim to prevent the emergence of novel influenza viruses in human populations through reassortment.

9.11.4.3.2 Alt-GoF Benefits to Pandemic Risk Assessments and Downstream Decision-Making for Pandemic Preparedness

Pandemic risk assessments of circulating animal influenza viruses inform decision-making about whether and how to invest in pre-pandemic vaccine development and other pandemic preparedness initiatives.

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[788] (2015q) Interviews with researchers at the National Wildlife Health Center (United States Geological Survey, Department of the Interior).

The genomic variation element of the IRAT includes consideration of reassortment, which may be informed by GoF studies that proactively assess the phenotypic consequences of reassortment events. In addition to genomic variation, several other types of information related to the properties of the virus are considered in the risk assessment: phenotypic data (i.e., transmissibility and virulence in ferrets), epidemiological data (i.e., the number and severity of human infections), and ecological data (i.e., factors related to infections in animals). In general, these factors are more important than the genomic variation risk element, in particular epidemiological and virologic data. However, a major drawback associated with these two data sources is that when novel viruses first emerge in human populations, epidemiological data may be scant and virus shipping delays will delay the generation of virologic data.

9.11.5 Comparison and Analysis of the Potential Benefits of GoF Approaches Versus Alt-GoF Approaches

9.11.5.1 Scientific Knowledge

GoF approaches are uniquely capable of *proactively* assessing the potential for *any* two influenza viruses to reassort, as well as for comprehensively evaluating the viability of various gene combinations. Notably, the outcomes of forced laboratory reassortment events may provide limited insight into the likelihood that such reassortment events will occur in nature, as natural reassortment depends on complex factors such as the rate of co-infection and the distribution of genetically compatible viruses (which are unknown). In addition, the relevance of this information for human populations depends on the suitability of animal models. Although surveillance-based approaches can provide broad insight into of the prevalence and distribution of reassortment viruses in different host populations, their utility is severely limited by the quality and availability of surveillance data. Similarly, the analysis of humans or animal isolates during co-infection is an unreliable method for determining the reassortment potential and genetic compatibility of two viruses, and opportunities for such studies are rare. The use of replication incompetent viruses is a promising approach for assessment of the genetic compatibility and reassortment potential between two viruses, but this system is not commonly used for this purpose and requires further validation. Moreover, it cannot capture the complex selection pressures observed *in vivo* and may not translate to mechanisms of reassortment in humans. Although the use of *in vitro* virus free systems is useful from an initial screening approach, results may not be recapitulated during the complete viral life cycle.

9.11.5.2 Surveillance

Both GoF and alt-GoF approaches provide information about the phenotypic properties of reassortant viruses detected through surveillance, which can inform analysis of their potential risks to human populations. The proactive nature of GoF studies facilitates more rapid assessment of surveillance data, but results may not translate to the strains observed in nature. In contrast, alt-GoF approaches provide more relevant information by directly studying the surveillance strains of interest but generate information after strains have been detected and require a viral isolate or high-quality genetic data for synthetic reconstruction of the virus.

Notably, the benefit of using experimental data about reassortant viruses (both GoF and alt-GoF) to aid the interpretation of surveillance data is severely constrained by the quality and availability of existing genetic surveillance data. Reassortment events are most commonly identified through individual phylogenetic analysis of each viral gene segment to identify its origin and ancestry.[769] This requires full genome sequences and large sequence databases for effective determination of phylogenetic ancestry,

[769] Steel J, Lowen AC (2014) Influenza A virus reassortment. *Current topics in microbiology and immunology* 385: 377-401

which are not always available, particularly for influenza viruses isolated from animal reservoirs.[790] Given these limitations, GoF and alt-GoF approaches to study reassortment currently provide minimal benefits to the interpretation of surveillance data. Full realization of their potential benefits will require significant expansion of genetic surveillance for reassortant viruses.

9.11.5.3 Decision-Making in Public Health Practice and Policy

9.11.5.3.1 Benefits to Risk Mitigation Activities That Aim to Prevent the Emergence of Reassortant Viruses in Nature

GoF studies that proactively study the reassortment potential between human seasonal viruses and animal viruses that have not yet caused human infections may help to prioritize risk communication and risk mitigation measures that aim to limit cross-species interactions that would provide opportunities for co-infection. These data also provide an evidence base for risk mitigation messaging that may increase buy-in among the target population. Alternative approaches can provide insight into the ecological factors that drive reassortment in nature, which is also needed to refine prioritization of risk communication and mitigation activities.

As environmental conditions that provide opportunities for co-infections with a human seasonal virus and an animal virus that has caused human infections are already of high concern, reassortment studies involving these viruses are unlikely to further increase preventive measures that are already in place.

9.11.5.3.2 Benefits to Pandemic Risk Assessments and Downstream Decision-Making for Pandemic Preparedness

Pandemic risk assessments of circulating animal influenza viruses inform decision-making about how to invest in public health preparedness activities for influenza pandemics, particularly development of pre-pandemic vaccines. The genomic variation risk element considered during pandemic risk assessments may be informed by GoF studies involving reassortment. In general, the genomic variation risk element is of low to intermediate importance relative to other factors considered in the risk assessment, in particular epidemiologic and virologic factors. However, corroboration of phenotypic data adds value to the assessment by increasing certainty in downstream decisions. Furthermore, GoF data plays a relatively more important role when novel viruses first emerge in human populations, when epidemiological data are likely to be scant and virus shipping delays will delay the generation of virologic data. In this case, the ability to evaluate risk based on genetic sequence data enables a rapid risk assessment, which can provide a head start on downstream response activities that would be valuable in the event of a pandemic.

9.12 Evaluation of the Quantitative Benefits of GoF Research

This section quantitatively explores the benefit of GoF and alt-GoF experiments that influence the availability of influenza vaccines during seasonal influenza epidemics and influenza pandemics. These benefits are briefly summarized below and are described in detail in the relevant GoF phenotype section

[790] Vincent A *et al* (2014) Review of influenza A virus in swine worldwide: a call for increased surveillance and research. *Zoonoses and public health* 61: 4-17

9.12.1 Overview of GoF and Alt-GoF Benefits Subject to Quantitative Analysis

9.12.1.1 GoF Experiments That Enhance Virus Production

GoF approaches that enhance virus production are currently used to produce egg- and cell-based influenza vaccines, which comprise over 99% of influenza vaccines produced annually in the US. Eliminating GoF approaches from the current vaccine production process would likely result in the inability to produce vaccine or the production of completely ineffective vaccines (due to poor vaccine match), as no alternative approaches can supplant the use of GoF approaches in the near-term.

GoF approaches that enhance virus production can also improve the current influenza vaccine production process. Specifically, GoF-derived improvements to the yields of vaccine viruses will increase the rate of bulk antigen production, thereby shortening vaccine production timelines. The production of influenza vaccines is highly optimized, such that current production capacities of eggs, the medium used for the majority of flu vaccine production, are at or near maximum levels. As a result, benefits derived from increasing vaccine virus yields primarily benefit vaccines based on viruses that grow poorly in eggs, such as the 2009 H1N1 pandemic virus. That is, incorporating insights from GoF research into those initially low-yield vaccine viruses could boost their production to "normal" levels. This improvement will lead to faster vaccine availability during future pandemics caused by viruses that have naturally low yields in eggs.

9.12.1.2 GoF Experiments That Enhance Infectivity, Transmissibility, and Virulence in Representative Animal Models

GoF approaches that enhance the infectivity, transmissibility, and virulence of influenza viruses in representative animal models also have potential to improve vaccine availability during a pandemic. Specifically, these GoF approaches strengthen the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence, which inform pandemic risk assessments of circulating animal influenza viruses. These assessments guide downstream decision-making about investments in pre-pandemic vaccine development, namely decisions about whether to develop candidate vaccine viruses (CVVs), develop a vaccine seed strain, produce clinical lot material, conduct clinical trials, and stockpile bulk antigen. In the event that a similar virus emerges to cause a pandemic, each of these preparative steps will shorten the time needed for large-scale production of that vaccine. Developing pre-pandemic CVVs, vaccine seed strains, and conducting clinical trials to determine the dosage, need for adjuvants, and other dosing parameters will eliminate steps from the production process, and manufacturers' experience working with the vaccine strain will streamline the subsequent production process. These improvements will translate to faster vaccine availability during a pandemic.

9.12.1.3 Alternative Approaches That Influence the Availability of Vaccines

Alternative approaches also have potential to increase the availability of influenza vaccines during a pandemic. Several alternative approaches can shorten production timelines for strain-specific influenza vaccines. First, the development of modified host cell lines that permit higher levels of virus replication increases the rate of bulk antigen production. Second, incorporating adjuvants into existing egg- and cell-based vaccines enables a smaller quantity of antigen to be used in each vaccine dose, thereby shortening the overall production timeline. Third, new, virus-free vaccine platforms, such as recombinant vaccines, have shorter production timelines than egg- and cell-based vaccines. Due to regulatory barriers, none of these alternatives have potential to influence vaccine production timelines in the near term, but each has potential to shorten production timelines in the intermediate- to long-term.

9.12.2 Overview of GoF Benefits Not Subject to Quantitative Analysis

9.12.2.1 Influenza Viruses

Other benefits of GoF research involving influenza viruses are not amenable to a meaningful quantitative analysis.

Approaches within two phenotypic categories (enhanced virus production and evasion of existing natural or induced adaptive immunity) have potential to improve the efficacy of seasonal flu vaccines. GoF approaches that enhance virus production can shorten vaccine production timelines, enabling selection of strains closer to the start of flu season, which will increase the likelihood that the "correct" strains are chosen resulting in well-matched vaccines. GoF approaches that lead to evasion of existing natural or induced adaptive immunity have potential to improve strain selection capabilities through several different mechanisms, which will similarly increase the likelihood that vaccines are well-matched to circulating strains at their time of deployment. The degree to which either advance will improve the likelihood of vaccine match is highly uncertain. Furthermore, the relationship between vaccine match and vaccine efficacy for a given flu season is complex, arising not only from the antigenic relationship between the vaccine strain and the dominant circulating strain but also historical factors such as the antigenic relationship between the dominant and recently circulating strains, vaccine coverage during the current and past flu seasons, and other factors. Thus, determining how an assumed increase in vaccine match translates to an increase in vaccine efficacy is also subject to considerable uncertainty. Given these uncertainties, quantitatively assessing the benefits of GoF improvements to vaccine efficacy in a meaningful way is not possible.

Approaches within several phenotypic categories (evasion of vaccines in development, enhanced virulence, and evasion of therapeutics) may benefit the development of novel vaccines and therapeutics. Exactly which novel medical countermeasures these studies may lead to is unknowable, so, even though the benefit of novel countermeasures could be assessed parametrically, the advent of any countermeasure with a specific property could not be tied directly to one of these GoF studies.

Finally, GoF studies involving reassortment may stimulate implementation of activities that aim to prevent the emergence of novel flu viruses in human populations. Whether these activities prevented the emergence of a pandemic virus is unknowable, thus this benefit was not quantified either.

9.12.2.2 Coronaviruses

The benefit associated with novel countermeasures against the SARS and MERS coronaviruses was not assessed because two counterfactuals must be assumed. Firstly, no pandemic of these diseases has occurred due to their susceptibility to public health control measures and spontaneous social distancing, and so the effects of notional countermeasures on mitigating a pandemic have doubtful relevance. Secondly, the geographic and temporal origins of the next outbreak of a novel strain of coronavirus are unpredictable, and therefore, even if the medical countermeasures existed, the supply of these countermeasures and the ability of the local public health system to distribute them is unknowable. For this reason, the magnitude of the benefit of medical countermeasures to controlling a local outbreak caused by a novel coronavirus is also subject to irreducible uncertainty.

9.12.3 Benefit Associated With Seasonal Influenza Vaccine

As described above, eliminating GoF from current vaccine production processes is likely to result in production of a completely ineffective influenza vaccine due to poor vaccine match or the inability to produce influenza vaccine. Figure 9.4 shows the number of deaths suffered in a typical seasonal influenza

outbreak given normal production and administration of vaccine compared to the complete absence of an effective vaccine. Although administration of seasonal influenza vaccine doses begins just prior to the start of "influenza season," many influenza infections exist in the US before this time and the overall predictions of deaths suffered is sensitive to how many infections are presumed to exist at this point. The figures below presume that either 100, 1,000 or 10,000 people infected with seasonal influenza exist prior to the onset of the season. Here, parameter values were chosen to illustrate models whose results match those seen for average seasonal flu outbreaks in the USA, to more closely illustrate the predicted benefits of seasonal flu vaccines. No matter which assumption is made, the lack of a vaccine significantly exacerbates the outbreak, increasing the number of deaths by ten to 100 fold. The effect observed is due not only to the protection of vaccinated individual from infection, but also greatly reduced case numbers overall due to herd immunity dampening the outbreak. This finding demonstrates that any measures that imperil vaccine production could have a significant and real cost.

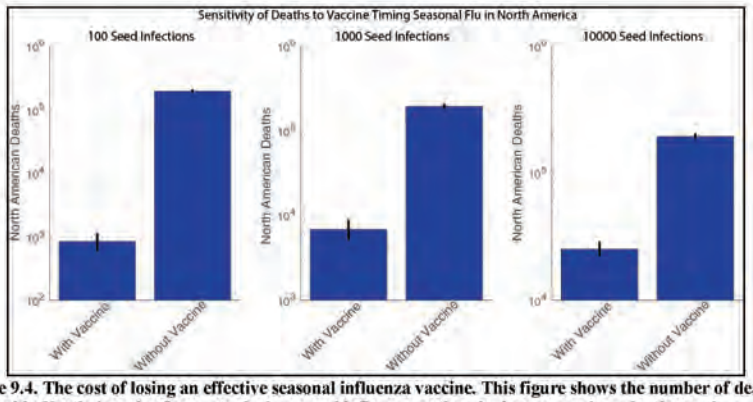

Figure 9.4. The cost of losing an effective seasonal influenza vaccine. This figure shows the number of deaths suffered in North America from a typical seasonal influenza outbreak given normal production and administration of the influenza vaccine or in the absence of the vaccine. The three panels show the number of deaths predicted if 100, 1,000 or 10,000 cases of influenza exist in North America at the time influenza vaccines begin to be administered just prior to the start of "influenza season". The R_0, case fatality rate, community mitigation strength, vaccine efficacy, and antiviral distribution values were fixed at single values illustrating an average flu season in the USA, and vaccine distribution was started immediately after the simulations began. Lines represent the middle 80% of the results across all remaining varied parameters.

9.12.4 Benefit Associated with Pandemic Influenza Vaccine

Unlike seasonal influenza, outbreaks of pandemic influenza are currently unpredictable. Because of this lack of warning, the length of the production cycle of vaccine to mitigate the pandemic is critical. The production timeline of a pandemic influenza vaccine influences how quickly after the outbreak is detected that the vaccine will be available. Figure 9.5 explores the relationship of the timing of the availability of pandemic influenza vaccine and deaths in North America. The particular strain modeled has a death rate and transmissibility that exceeds that of the 2009 pandemic strain (to better reflect other pandemic strains). These data can be used to evaluate the quantitative benefits of several GoF and alt-GoF approaches that influence the production timelines of influenza vaccines: (1) GoF approaches that are currently used for vaccine production, which are needed to maintain the current ability to produce pandemic influenza vaccines, (2) GoF approaches that shorten existing vaccine production timelines, which have potential to improve vaccine availability in the near-term, and (3) alt-GoF approaches that

shorten vaccine production timelines (i.e., development of modified cell lines, use of adjuvants for dose sparing, and development of new, virus-free vaccine platforms), which have potential to improve vaccine availability in the intermediate- to long-term.

As described above, eliminating GoF approaches from existing vaccine production practices would likely result in production of a completely ineffective vaccine or in the inability to produce a vaccine. The consequences of having no vaccines available during a pandemic, relative to vaccines that can be deployed on current production timescales, are illustrated through comparison of the "current typical" and "never" time points on the graphs in Figure 9.5. As demonstrated, the benefit at mitigating the outbreak for the vaccine depends heavily on how the public reacts to the outbreak. On the right, if the public barely changes its behavior during the outbreak, current vaccine production timelines are too slow to prevent a significant number of deaths, thus the difference between deploying vaccines on current timescales and never deploying vaccines is minimal. However, if the public reacts strongly and reduces their usual contacts by 25% (left panel, community mitigation 0.25), then any delay of vaccine production could increase the number of deaths expected. The inability to deploy vaccine would increase the number of deaths, relative to deployment of vaccine on typical timescales, 1.2 to six-fold depending on how rapidly the rapidly the pandemic is detected (indicated by the number of seed infections at the start of vaccine production).

GoF and alt-GoF approaches also have potential to shorten vaccine production timelines, which would enable deployment of vaccine earlier during a pandemic. However, the extent to which alternative approaches could shorten vaccine production timelines in the future is uncertain. If the public does not change their behavior during a pandemic, production timelines must be shortened by more than six weeks to significantly reduce the number of deaths (i.e., the production timeline must be faster than the 'current optimal' timeline). If the public reduces their usual contacts by 25%, then any reduction in the time needed to produce vaccines would reduce the number of deaths during a pandemic.

As described above, GoF approaches that enhance virus production will primarily aid production of vaccines based on "slow" growing viruses, allowing these vaccines to be produced on closer-to-typical timescales. Thus, comparison of the "current slow" and "current optimal" time points on the graphs in Figure 9.5 provides an estimate of the scale of this benefit using a vaccine with mean efficacy during a pandemic with median R_0 and case fatality rate twice that of the seasonal outbreak above. If the public does not change their behavior during the pandemic (right graph), this improvement to production would have minimal impacts on the number of deaths because the typical production timeline is too slow for vaccination to significantly mitigate the consequences of a pandemic. However, if the public reduces contacts by 25% (left graph), the number of deaths predicted will decrease by roughly 30%.

Implementing one or more stages of the pre-pandemic vaccine development pipeline, influenced by GoF approaches that enhance the infectivity, transmissibility, and virulence of influenza viruses, could also shorten vaccine production timelines during a pandemic. Even if the public does not change their behavior during the pandemic, shortening production timelines by nine weeks could reduce the number of deaths by 15 to 30% (compare "current optimal" to "9 weeks faster" time points, right graph). If the public does reduce contact rates, this improvement to production would decrease the number of deaths by 60 to 70%, which would save more than 100,000 lives in a high mortality outbreak.

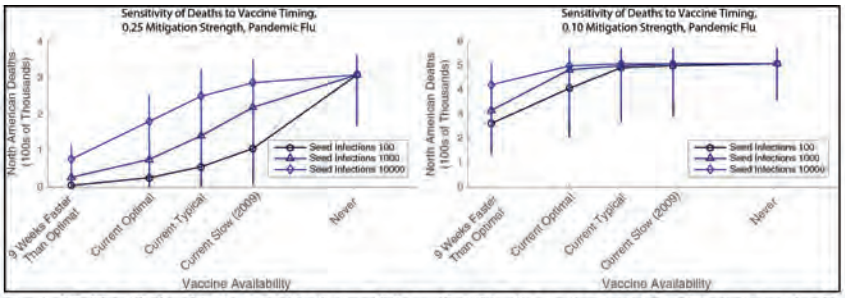

Figure 9.5. The relationship between the timing of the availability of a vaccine against an emergent pandemic influenza strain and deaths suffered in North America for two different values of community mitigation strength. Results are shown for a vaccine of mean efficacy and an outbreak with a median R0and case fatality rate twice that of the seasonal outbreak depicted above. The right panel shows results if the public barely chances its behavior (10% fewer contacts) whereas the left panel shows the results if the public reduces its contacts by 25% for the duration of the outbreak. The three lines on each graph show the results if the vaccine production process starts when there are 100, 1,000 or 10,000 cases in North America.

9.13 Likelihood of GoF Strains Arising in Nature

9.13.1 Summary

GoF experiments that enhance the transmissibility or virulence of influenza viruses, that lead to evasion of existing natural or induced adaptive immunity, and that lead to evasion of therapeutics are pursued to gain insight into the mechanisms underlying those phenotypic changes and to generate information that can benefit public health. Both the potential benefits of those experiments, as well as the public health risks of *not* conducting the experiments, depend on the likelihood that the phenotypic changes observed in the laboratory will occur in nature. Antigenic drift of seasonal influenza viruses and evolution of antiviral resistance (in both seasonal and animal influenza viruses) both occur regularly in nature. Influenza viruses exhibit a wide spectrum of virulence in humans. Notably, the 1918 H1N1 pandemic virus caused a case fatality rate several orders of magnitude higher than seasonal influenza viruses, demonstrating that viruses with high virulence can emerge to cause pandemics.

Animal influenza strains are not known to have directly evolved the capacity for efficient transmission in humans. In contrast, the fact that the four influenza pandemics of the past century were caused by reassortant viruses definitively demonstrates that enhanced transmissibility in humans can arise through reassortment between human seasonal and animal influenza strains, including the generation of viruses of HA subtypes that are "novel" to the human population (e.g., the 1957 H2N2 pandemic virus and the 1968 H3N2 pandemic virus).

Animal influenza viruses that continue to infect humans, in particular swine H3N2v viruses and avian influenza H5N1, H7N9, and H9N2 viruses, do not efficiently infect or transmit in people. However, some of these viruses share phenotypic properties of viruses that do efficiently transmit in humans, including the ability to transmit via the respiratory route in ferrets and the ability to binding "human-like" sialic acid receptors, and computational modeling suggests that the set of adaptive mutations needed to confer the capacity for airborne transmission in mammals to H5N1 viruses can accrue during a single round of transmission in a human host. The evolutionary implications of these findings–whether these viruses are likely or unlikely to directly evolve the capacity for efficient transmission in humans– are unknowable,

given the small number of pandemics from which to draw lessons about the natural evolution of human transmissibility. Critically, the fact that fully avian influenza strains have adapted to efficiently transmit between dogs definitively demonstrates that cross-species adaptation of avian viruses to mammals is possible. Furthermore, lessons learned from experiments that enhance the transmissibility of fully avian or swine strains may be generalizable to mixed-species reassortant strains, thus their value does not depend on whether the strains under study are likely to directly evolve enhanced transmissibility.

9.13.2 Introduction

Gain of Function (GoF) experiments can be classified into two broad categories based on the purpose and outcomes of the approach: (1) experiments that generate tools for scientific or public health use and (2) experiments that enhance scientific understanding of virus behavior. The "tool" category of approaches includes those that generate knowledge or products for use in vaccine production, such as high-yield candidate vaccine viruses (CVVs) and knowledge about molecular markers that improve CVV growth and those that adapt viruses for growth in mice or ferrets to generate animal models. These approaches are not designed to generate or study phenotypes that are likely to occur in nature, and their benefits derive solely from use of the information/tools for further scientific study or for MCM development/production.

The second category of GoF experiment generates scientific information that enhances the understanding of virus physiology and behavior, which improves scientific knowledge and may additionally benefit public health. This category includes GoF approaches that enhance the infectivity and transmissibility of animal influenza viruses in mammals, that enhance the pathogenicity of influenza viruses in appropriate animal models, that lead to evasion of existing natural or induced immunity, that lead to evasion of therapeutics, and that involve reassortment between two different virus strains. Findings from these approaches demonstrate what is *possible* for viral physiology and behavior in model systems and in a laboratory environment. Importantly, the scientific relevance of this information and its utility for public health depends on whether the phenotypes under study are likely to arise in nature. For example, using information about molecular markers of mammalian adaptation in avian influenza viruses to prioritize pandemic preparedness investments may be inappropriate if avian influenza viruses are unlikely to evolve to efficiently infect humans in nature. As efforts to study these phenotypes aim to directly or indirectly aid efforts to mitigate the public health consequences of seasonal influenza epidemics and influenza pandemics, the likelihood of GoF phenotypes arising in nature also speaks to the risk of *not* pursuing GoF research.

To provide context for our evaluation of the benefits of this research, this report will evaluate the likelihood that the four GoF phenotypes listed in the paragraph above will arise in nature. Within each phenotypic category, we first briefly review relevant GoF studies and results. Next, we draw upon several types of evidence to evaluate whether the phenotype is likely to arise in nature, namely characterization of wildtype viruses, epidemiological studies, and computational modeling approaches.

9.13.3 Evasion of Existing Natural or Induced Immunity (Antigenic Drift)

GoF approaches in this phenotypic category experimentally induce antigenic drift of seasonal influenza viruses in the laboratory through serial passage of viruses in the presence of cognate antibodies or through targeted mutagenesis to introduce mutations expected to confer antigenic change. These approaches provide insight into the mechanisms underlying antigenic drift and also generate information that may benefit antigenic surveillance of seasonal influenza viruses and strain selection for seasonal flu vaccines. Within the Framework definition of GoF, this phenotypic category includes experiments that generate novel antigenicity-altering amino acid substitutions, which have not yet been observed in nature, as well as those that test the phenotypic consequences of particular amino acid substitutions found in wild type

strains identified through surveillance. The likelihood that the phenotypic changes observed in the former type of experiment (i.e., forcing antigenic drift of currently circulating influenza strains) is of interest for this report.

Since the emergence of the seasonal H1N1 and H3N2 strains of influenza in human populations (i.e., following the 1918 H1N1 pandemic and the 1968 H3N2 pandemic), both strains have drifted antigenically in nature. For example, the H3N2 strain underwent ten antigenic changes (termed antigenic cluster transitions) between its emergence in 1968 and 2004, typically drifting every two to four years.[791] The H1N1 strain has also drifted over time, exhibiting 16 antigenic changes between 1918 and 2008, with each antigenic cluster circulating for one to ten years prior to drift.[792] Antigenic variants of the 2009 H1N1 pandemic strain have been detected in nature but have not yet become widespread, such that the H1N1 component of the seasonal flu vaccine has not changed since the emergence of the virus in 2009.[793,794,795] These observations definitively demonstrate that antigenic drift of currently circulating influenza viruses, as induced through GoF experiments, occurs regularly in nature.

9.13.4 Evasion of Therapeutics

GoF approaches in this phenotypic category experimentally generate antiviral-resistant strains through serial passage of viruses in the presence of sub-inhibitory concentrations of therapeutic or through targeted genetic modification to introduce mutations expected to confer antiviral resistance. These approaches aim to gain insight into the mechanistic basis of antiviral resistance. An additional goal is the identification of mutations that confer antiviral resistance for use in surveillance, which influences therapeutic recommendations for seasonal influenza infections and pandemic preparedness initiatives for animal influenza viruses. Within the Framework definition of GoF, this phenotypic category includes experiments that confer antiviral resistance to particular strains that have not yet exhibited resistance in nature as well as those that test the phenotypic consequences of mutations observed in wild type antiviral resistant strains. As above, the likelihood that the phenotypic changes observed in the former type of experiment will arise in nature is of interest for this report.

Mutations that confer resistance to both classes of licensed influenza antivirals, the adamantanes and the neuraminidase inhibitors (NAIs), have arisen in nature. The adamantane class of antivirals, introduced into clinical practice in the early 1960s, were widely used as the primary treatment for influenza for 40 years. However, in the early 2000s, resistant strains emerged in nature, in particular strains carrying an S31N mutation in the M2 protein, and quickly rose to worldwide prominence across multiple strain sub-types.

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791 Smith DJ *et al* (2004) Mapping the Antigenic and Genetic Evolution of Influenza Virus. *Science* 305: 371-376

792 Liu M *et al* (2015a) Antigenic Patterns and Evolution of the Human Influenza A (H1N1) Virus. *Sci Rep* 5: 14171

793 Huang W *et al* (2015) Characteristics of oseltamivir-resistant influenza A (H1N1) pdm09 virus during the 2013-2014 influenza season in Mainland China. *Virol J* 12: 96

794 Makkoch J *et al* (2012) Whole Genome Characterization, Phylogenetic and Genome Signature Analysis of Human Pandemic H1N1 Virus in Thailand, 2009–2012. *PloS one* 7: e51275

795 Ramos AP *et al* (2013b) Molecular and phylogenetic analysis of influenza A H1N1 pandemic viruses in Cuba, May 2009 to August 2010. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* 17: e565-567

Specifically, the S31N mutation was identified in 0.4% of viruses in 1995 but its prevalence increased to 92% of viruses by 2006.[796,797] Widespread resistance persists, and the adamantanes are no longer recommended for treatment.[798]

Although resistance to NAIs is not yet widespread, resistance to one or multiple NAIs has been observed in wild type strains. Specifically, strains that are resistant to oseltamivir or zanamivir as well as strains that are resistant to both drugs have been observed in nature, including human seasonal strains (i.e., A/H1N1,[799] A/H3N2,[800] and B strains[801]) as well as animal influenza strains (e.g., H7N9).[802] In fact, resistance to oseltamivir in seasonal flu strains was widespread during the 2007 – 2008 and 2008 – 2009 seasons, and resistant strains continue to be sporadically detected.[803,804] NAI resistance has been linked to a variety of mutations, several of which were first discovered in the laboratory through GoF studies. For example, a GoF experiment discovered that the combination of H274Y and E119D mutations (N1 numbering) conferred pan-resistance to all three licensed NAIs (oseltamivir, zanamivir, and peramivir).[805] This set of mutations was later found to arise in an immunocompromised individual subjected to multiple NAI treatment regimens over a prolonged course of illness, with minimal effects on viral growth.[806]

Taken together, these observations definitively demonstrate that NAI resistance has evolved and is likely to continue to evolve in nature, and that particular antiviral resistance mutations identified through GoF studies have naturally arisen in human populations.

9.13.5 Enhanced Pathogenicity

GoF approaches in this phenotypic category experimentally generate more virulent viruses in representative model systems through serial passage of viruses in cells or animals or through targeted genetic modification to introduce traits expected to enhance virulence (including reassortment and targeted mutagenesis). These approaches aim to identify genetic and phenotypic traits underlying pathogenicity, which provides insight into basic virulence mechanisms and can inform pandemic risk assessments of circulating animal influenza viruses. Additionally, an improved understanding of how viruses cause disease provides a foundation for the development of new therapeutics, in particular therapeutics that protect against the severe disease observed during infection with highly pathogenic avian influenza (HPAI) viruses such as H5N1.

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796 Bright RA *et al* (2005) Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet* 366: 1175-1181

797 Bright RA *et al* (2006) Adamantane resistance among influenza A viruses isolated early during the 2005-2006 influenza season in the United States. *JAMA* 295: 891-894

798 CDC. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Update November 3, 2015. Accessed November 28, 2015.

799 Gubareva LV *et al* (2001) Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *J Infect Dis* 183: 523-531

800 Abed Y *et al* (2006) Impact of neuraminidase mutations conferring influenza resistance to neuraminidase inhibitors in the N1 and N2 genetic backgrounds. *Antiviral therapy* 11: 971-976

801 Fujisaki S *et al* (2012) A single E105K mutation far from the active site of influenza B virus neuraminidase contributes to reduced susceptibility to multiple neuraminidase-inhibitor drugs. *Biochem Biophys Res Commun* 429: 51-56

802 Sleeman K *et al* (2013) R292K substitution and drug susceptibility of influenza A(H7N9) viruses. *Emerging infectious diseases* 19: 1521-1524

803 Dharan NJ *et al* (2009) Infections with oseltamivir-resistant influenza A(H1N1) virus in the United States. *JAMA* 301: 1034-1041

804 Hauge SH *et al* (2009) Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. *Emerg Infect Dis* 15: 155-162

805 Baek YH *et al* (2015) Profiling and characterization of influenza virus N1 strains potentially resistant to multiple neuraminidase inhibitors. *Journal of virology* 89: 287-299

806 L'Huillier AG *et al* (2015b) E119D Neuraminidase Mutation Conferring Pan-Resistance to Neuraminidase Inhibitors in an A(H1N1)pdm09 Isolate From a Stem-Cell Transplant Recipient. *J Infect Dis*

A wide range of virulence has been observed in influenza strains that have infected humans, as detailed in Table 9.3.

Table 9.3. Viruses Display Different Levels of Virulence

Virus	CFR
Pandemic H3N8 ("Russian Flu", 1889)	0.1 – 0.28%[807]
Pandemic H1N1 ("Spanish Flu", 1918)	2-3%[808]
Pandemic H2N2 ("Asian Flu", 1957)	~0.1%[809,810]
Pandemic H3N2 ("Hong Kong Flu", 1968)	~0.1%[811,812]
Pandemic H1N1 (2009)	0.4%[813,814]
H5N1 outbreaks	53%[815]
H7N9 outbreaks	40%[816]
Seasonal strains	0.01 – 0.5%[817]

Notably, there is a 100- to 1000-fold difference in the estimated case fatality rate (CFR) for seasonal influenza viruses versus the 1918 H1N1 pandemic strain. Although the difference in observed CFR may be partly explained by poor public health knowledge and capabilities in 1918 relative to the modern era, experimental studies in ferrets also demonstrate that the 1918 H1N1 strain is highly pathogenic relative to modern H1N1 viruses.[818] Other pandemic strains (1957 H2N2, 1968 H3N2, and 2009 H1N1) have also exhibited higher virulence than seasonal influenza strains, albeit to a lesser degree than the 1918 H1N1 virus. Furthermore, H5N1 and H7N9 avian influenza strains that sporadically infect humans cause severe, disseminated disease, exhibiting distinct cell and tissue tropism than human seasonal viruses. How

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807 Valleron A-J *et al* (2010) Transmissibility and geographic spread of the 1889 influenza pandemic. *PNAS* 107: 8778-8781

808 "Report of the Review Committee on the Functioning of the International Health Regulations (2005) in relation to Pandemic (H1N1) 2009," *World Health Organization*, accessed August 25, 2015, http://apps.who.int/gb/ebwha/pdf_files/WHA64/A64_10-en.pdf

809 Li FC *et al* (2008) Finding the real case-fatality rate of H5N1 avian influenza. *Journal of epidemiology and community health* 62: 555-559

810 Taubenberger JK, Morens DM (2006) 1918 Influenza: the mother of all pandemics. *Emerging infectious diseases* 12: 15-22.

811 Li FC *et al* (2008) Finding the real case-fatality rate of H5N1 avian influenza. *Journal of epidemiology and community health* 62: 555-559

812 Taubenberger JK, Morens DM (2006) 1918 Influenza: the mother of all pandemics. *Emerging infectious diseases* 12: 15-22.

813 Vaillant L *et al* (2009) Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 14

814 Fraser C *et al* (2009) Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings. *Science* 324: 1557-1561

815 WHO. Cumulative number of confirmed human cases for avian influenza A(H5N1)reported to WHO, 2003-2015. http://www.who.int/influenza/human_animal_interface/EN_GIP_20151113cumulativeNumberH5N1cases.pdf?ua=1. Last Update November 13, 2015. Accessed November 28, 2015.

816 WHO. Influenza at the human-animal interface. Summary and assessment as of 17 July 2015. http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_17_July_2015.pdf. Last Update Accessed November 28, 2015.

817 Meltzer MI *et al* (2015) Standardizing scenarios to assess the need to respond to an influenza pandemic. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 60 Suppl 1: S1-8

818 Bootsma MCJ, Ferguson NM (2007) The effect of public health measures on the 1918 influenza pandemic in U.S. cities. *PNAS* 104: 7588-7593

virulence would change if these strains were to adapt to efficiently infect and transmit in humans is unknown.

Critically, it is not possible to predict the virulence and pathogenesis mechanisms of the next pandemic influenza strain. However, the fact that past pandemic strains have exhibited higher levels of virulence than seasonal strains, that 1918-like avian viruses are currently circulating in wild bird populations, and that human infections with some H5 and H7 strains causes severe disease suggest that a virulent pandemic strain *could* naturally emerge.[819] This possibility lends support to the study of virulence using GoF approaches, as these studies aim to generate knowledge that improves preparedness for pandemics caused by highly virulent influenza strains.

9.13.6 Mammalian Adaptation and Enhanced Transmission in Representative Animal Models

GoF approaches in this phenotypic category experimentally generate viruses with enhanced infectivity and transmissibility in representative animal models through serial passage of viruses in animals and/or through targeted genetic modification to introduce traits expected to enhance infectivity or transmissibility. These experiments aim to understand whether and how animal influenza viruses can adapt to efficiently infect and transmit in humans, which provides insight into the mechanisms underlying mammalian adaptation and transmissibility. This information also facilitates monitoring of the pandemic risk posed by animal influenza viruses circulating in nature, which informs development of vaccines and other pandemic preparedness initiatives that seek to mitigate the public health consequences of a pandemic caused by animal-origin viruses. This phenotypic category includes experiments involving animal influenza viruses (e.g., HPAI H5N1) as well as experiments involving reassortment viruses comprised of gene segments from human seasonal and animal influenza viruses (e.g., an H5N1 reassortment strain comprised of an avian H5 gene and the remaining seven genes from the human pandemic H1N1 strain).[820,821] Experiments using both types of animal flu viruses have led to the generation of modified viruses that are capable of transmitting between appropriate animal models (guinea pigs, for contact transmission studies, or ferrets, for contact and airborne transmission studies). Specifically, mammalian-transmissible variants of avian influenza H5N1 and H7N1 strains have been generated in the laboratory, as well as mammalian-transmissible reassortment strains comprised of gene segments from human seasonal viruses and either avian influenza H5N1 or H9N2 strains.[822,823,824,825,826,827,828] (Of note, serial passaging and/or reassortment studies involving other avian influenza

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819 Watanabe T *et al* (2014a) Circulating avian influenza viruses closely related to the 1918 virus have pandemic potential. *Cell Host Microbe* 15: 692-705

820 Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

821 Imai M *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486: 420-428

822 ibid.

823 Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

824 Sutton TC *et al* (2014) Airborne transmission of highly pathogenic H7N1 influenza virus in ferrets. *Journal of virology* 88: 6623-6635

825 Wan H *et al* (2008) Replication and Transmission of H9N2 Influenza Viruses in Ferrets: Evaluation of Pandemic Potential. *PloS one* 3
Li X *et al* (2014b) Genetics, receptor binding property, and transmissibility in mammals of naturally isolated H9N2 Avian Influenza viruses. *PLoS Pathog* 10: e1004508

826 Chen L-M *et al* (2012) In vitro evolution of H5N1 avian influenza virus toward human-type receptor specificity. *Virology* 422: 105-113

827 Zhang Y *et al* (2013a) H5N1 Hybrid Viruses Bearing 2009/H1N1 Virus Genes Transmit in Guinea Pigs by Respiratory Droplet. *Science* 340: 1459-1463

828 Sorrell EM *et al* (2009) Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus. *Proc Natl Acad Sci U S A* 106: 7565-7570

strains, such as serial passaging of H7N9 viruses, have not led to the generation of viruses with enhanced transmissibility.)[829]

Notably, experiments in this phenotypic category are distinct from others included in the Framework in that avian or swine viruses that efficiently infect and transmit in humans have not yet evolved in nature. While reassortant strains with genes of avian and/or swine origin have emerged to cause pandemics in human populations, neither reassortant nor fully animal-origin strains of the H5, H7, or H9 sub-types, which are thought to have the greatest pandemic potential of the avian influenza strains that have infected humans, have evolved the capacity for efficient human transmission. Given the caveats associated with translating laboratory results to nature, some have questioned whether the animal influenza strains used in these GoF studies could ever naturally acquire enhanced infectivity and transmissibility in humans. As mentioned above, the likelihood that this evolution could occur motivates the GoF studies and qualitatively speaks to the risk of not investing in research that aims to mitigate the effects of future pandemics caused by descendants of these viruses.

This section evaluates the likelihood that animal strains could evolve the capacity for efficient infection and transmission in humans through either the direct evolution and/or the reassortment pathway. Three types of evidence are reviewed: (1) epidemiological data about human infections with animal influenza viruses, (2) laboratory data about the characterization of wild type viruses, detected through surveillance, and (3) computational modeling of the capacity of wild type viruses to evolve mammalian transmissibility.

9.13.6.1 Epidemiological Data

Relevant epidemiological data includes incidence, severity, and patterns of infection in humans (and non-human mammals), as well as serological studies investigating population exposure to influenza viruses.

9.13.6.1.1 Cross-Species Adaptation Events Not Involving Humans

Although avian influenza (AI) viruses have not directly adapted to efficiently infect and transmit in humans, AI viruses have directly evolved to efficiently transmit between other mammals. Namely, an avian-origin H3N2 canine influenza virus emerged in dogs in the mid-2000s and is now circulating in dog populations of China and South Korea, and possibly Thailand.[830] Phylogenetic analysis revealed that canine adaptation involved both intrasubtypic and heterosubtypic reassortment events as well as the evolution of adaptive mutations. Isolated spillover events of avian influenza viruses in mammals have also been detected, similar to humans. For example, in 2004, a dog was found to develop high fever and lethargy following ingestion of duck carcasses. Necropsy revealed extensive H5N1 infection in the canine tissues.[831] In 2011, several New England harbor seals were found to be infected with an avian H3N8 virus that exhibited enhanced affinity for α2,6 receptors and was transmissible via respiratory droplets in ferrets.[832] Taken together, these examples demonstrate that avian influenza viruses have the capacity to infect and evolve efficient transmissibility in non-human mammals.

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[830] Zhu H *et al* (2015) Origins and Evolutionary Dynamics of H3N2 Canine Influenza Virus. *Journal of virology* 89: 5406-5418
[831] Songserm T *et al* (2006) Fatal Avian Influenza A H5N1 in a Dog. *Emerg Infect Dis* 12: 1744-1747
[832] Karlsson EA *et al* (2014) Respiratory transmission of an avian H3N8 influenza virus isolated from a harbour seal. *Nat Commun* 5

9.13.6.1.2 Cross-Species Adaptation Events Involving Humans

Numerous swine and avian influenza strains have infected humans, reviewed below. These data speak to the current capacity for circulating zoonotic influenza strains to infect and transmit in people.

Swine influenza strains H1N1v and H1N2v

Human infections with swine influenza strain H1N1v have been reported for decades, as far back as the 1930s.[833] However, since 2005, only 19 cases of H1N1v infections in the US have been reported to the CDC, leading to one fatality, and human to human transmission has not been documented.[834,835,836,837] Five non-fatal cases of human infection with swine influenza strain H1N2v have also been reported.[838,839] Both variant viruses cause symptoms similar to seasonal strains. H1N1v infections have been reported in several other countries, though in general surveillance for swine influenza infections is poor outside the US and Europe.[840,841] Swine farm workers have been shown to have higher HI antibody titers against H1N1 than the general population, suggesting that they are frequently exposed to H1N1 virus but experience asymptomatic or sub-clinical infections.[842]

Swine influenza H3N2v

The first human case of infection with H3N2v was reported in the United States in July 2011, although the virus was first detected in the US stock of pigs in 2010.[843] As of 2015, 353 human infections with H3N2v have been reported to the CDC, most of which occurred during outbreaks linked to agricultural fairs in Ohio and Indiana in 2012.[844,845,846] H3N2v illness is relatively mild; only 18 of the US patients were hospitalized and only one of those cases was fatal.[847] Two clusters of cases– three children in Iowa who visited the same health care provider and two children in West Virginia who attended the same day

833 Shope RE (1931) Swine Influenza : III. Filtration Experiments and Etiology. *J Exp Med* 54: 373-385

834 CDC. Reported Infections with Variant Influenza Viruses in the United States since 2005. http://www.cdc.gov/flu/swineflu/variant-cases-us.htm#table-infections. Last Update September 4, 2015. Accessed November 28, 2015.

835 Dacso CC *et al* (1984) Sporadic occurrence of zoonotic swine influenza virus infections. *Journal of clinical microbiology* 20: 833-835

836 Avian Flu Diary. http://afludiary.blogspot.com/2015/08/cdc-fluview-1-novel-h1n1v-case-reported.html. Last Update August 28, 2015. Accessed November 28, 2015.

837 Centers for Disease Control and Prevention. (2014a) Influenza Activity — United States, 2014-15 Season and Composition of the 2015–16 Influenza Vaccines. *Morbidity and Mortality Weekly Report*, Vol. 64, pp. 583-590.

838 Ibid.

839 CDC. Reported Infections with Variant Influenza Viruses in the United States since 2005. http://www.cdc.gov/flu/swineflu/variant-cases-us.htm#table-infections. Last Update September 4, 2015. Accessed November 28, 2015.

840 Niemcewicz M *et al* (2013) Acute respiratory distress syndrome (ARDS) in the course of influenza A/H1N1v infection–genetic aspects. *Ann Agric Environ Med* 20: 711-714

841 Calistri A *et al* (2011) Report of two cases of influenza virus A/H1N1v and B co-infection during the 2010/2011 epidemics in the Italian Veneto Region. *Virology Journal* 8: 502

842 Olsen CW *et al* (2002) Serologic Evidence of H1 Swine Influenza Virus Infection in Swine Farm Residents and Employees. *Emerging infectious diseases* 8: 814-819

843 Centers for Disease Control and Prevention. Seasonal Influenza (Flu): H3N2v and You. http://www.cdc.gov/flu/swineflu/h3n2v-basics.htm. Last Update August 2014. Accessed September 2014.

844 "Reported Infections with Variant Influenza Viruses in the United States since 2005 | Swine/Variant Influenza (Flu)," accessed August 26, 2015, http://www.cdc.gov/flu/swineflu/variant-cases-us.htm.

845 Greenbaum A *et al* (2015) Investigation of an Outbreak of Variant Influenza A(H3N2) Virus Infection Associated With an Agricultural Fair-Ohio, August 2012. *J Infect Dis*

846 Centers for Disease Control and Prevention (CDC), "Notes from the Field: Outbreak of Influenza A (H3N2) Virus among Persons and Swine at a County Fair--Indiana, July 2012," *MMWR. Morbidity and Mortality Weekly Report* 61, no. 29 (July 27, 2012): 561.

847 CDC. Case Count: Detected U.S. Human Infections with H3N2v by State since August 2011. http://www.cdc.gov/flu/swineflu/h3n2v-case-count.htm. Last Update September 4, 2015. Accessed November 28, 2015.

care and had no known contact with swine prior to symptom onset– suggest that H3N2v viruses are capable of limited human-to-human transmission.[848,849]

Avian influenza H5Nx Strains

Highly pathogenic avian influenza H5N1 first caused human infections in 1997, following a poultry outbreak in Hong Kong.[850] Since 2003, 844 cases, 449 of which were fatal, were reported to the WHO, representing a 53% case fatality rate.[851] Most H5N1 cases have been in countries with a high prevalence of backyard farming and active live poultry markets (LPMs), both providing opportunities for human exposure to avian viruses through infected poultry.[852] Several statistical models have attempted to estimate the R_0 of the H5N1 outbreaks, to determine whether the virus has the capacity for human-to-human transmission; however, different research groups have generated drastically different estimates. One group estimated an R_0 value of 1.14, which meets criteria for self-sustaining transmission, but others estimate the R_0 of H5N1 closer to 0.2.[853, 854] One major epidemiological case study in Vietnam gathered strong evidence to suggest human-to-human transmission of H5N1, while other studies evaluating H5N1 infection patterns in family clusters suggested the converse.[855,856] Thus, the extent to which spillover H5N1 viruses have any capacity for human-to-human transmission remains uncertain (and may vary by strain). A recent seroepidemiological study in Egypt, a country with a large number of documented human H5N1 cases, suggested that the prevalence of H5N1 infection is approximately 2% among Egyptians exposed to poultry, though few of those exposed had experienced clinical symptoms of infection.[857] These data suggest that most H5N1 infections are asymptomatic or sub-clinical, such that the "true" case fatality rate is much lower than that previously suggested based on the outcomes of the severe cases that are reported to the WHO.

Only one other avian A/H5 strain has caused human infections– H5N6, which is also highly pathogenic in poultry, has caused one fatal infection.[858]

Taken together, avian influenza H5N1 is capable of causing severe infections in humans, but epidemiological and seroepidemiological data suggests that the virus is poorly able to infect and transmit in humans.

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848 Centers for Disease C, Prevention (2011) Limited human-to-human transmission of novel influenza A (H3N2) virus--Iowa, November 2011. *MMWR Morb Mortal Wkly Rep* 60: 1615-1617

849 Centers for Disease C, Prevention (2012) Update: Influenza A (H3N2)v transmission and guidelines - five states, 2011. *MMWR Morb Mortal Wkly Rep* 60: 1741-1744

850 WHO. Avian Influenza Fact Sheet. http://www.who.int/mediacentre/factsheets/avian_influenza/en/. Last Update September 2014. Accessed November 28, 2015.

851 WHO. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2015. http://www.who.int/influenza/human_animal_interface/EN_GIP_20151113cumulativeNumberH5N1cases.pdf?ua=1. Last Update November 13, 2015. Accessed November 28, 2015.

852 WHO. Avian Influenza Fact Sheet. http://www.who.int/mediacentre/factsheets/avian_influenza/en/. Last Update September 2014. Accessed November 28, 2015.

853 Ferguson NM *et al* (2004) Public Health Risk from the Avian H5N1 Influenza Epidemic. *Science* 304: 968-969
Aditama TY *et al* (2012) Avian influenza H5N1 transmission in households, Indonesia. *PloS one* 7: e29971

854 Yang Y *et al* (2007) Detecting human-to-human transmission of avian influenza A (H5N1). *Emerging infectious diseases* 13: 1348-1353

855 Tran TH *et al* (2004) Avian influenza A (H5N1) in 10 patients in Vietnam. *N Engl J Med* 350: 1179-1188

856 Olsen SJ *et al* (2005) Family Clustering of Avian Influenza A (H5N1). *Emerging infectious diseases* 11: 1799-1801

857 Gomaa MR *et al* (2015) Avian influenza A(H5N1) and A(H9N2) seroprevalence and risk factors for infection among Egyptians: a prospective, controlled seroepidemiological study. *J Infect Dis* 211: 1399-1407

858 Pan M *et al* (2015) Human infection with a novel highly pathogenic avian influenza A (H5N6) virus: Virological and clinical findings. *J Infect*

Avian influenza H7 strains

Several subtypes of avian H7Nx have caused human infections: H7N2, H7N3, H7N7, and H7N9. Six cases of H7N2 infection have been reported worldwide, most in patients who had been in close contact with infected poultry prior to their infections.[859,860] Although several patients were hospitalized, all recovered from their infections. Several cases of H7N3 infection have also been documented in poultry workers following contact with infected flocks; most experienced mild or sub-clinical infections, and all patients recovered.[861,862] The first documented human H7N7 infection occurred in the UK in 1996, in a woman who contracted the virus while cleaning her poultry shed. She exhibited mild symptoms and fully recovered.[863] The 2002– 2003 human H7N7 outbreak in the Netherlands, which occurred as the result of outbreaks in poultry populations, was the first non-H5N1 avian influenza outbreak in humans. Over 1,000 people had subclinical indications, 86 people were infected, including poultry workers and several of their family members, and at least one person died from infection complications.[864] H7N7 infections were again documented in three poultry workers following a 2013 outbreak in Italy, all of who displayed mild symptoms and recovered.[865]

As of November 2015, 683 people have been confirmed with a novel reassortant H7N9 virus, and 271 have died from the infection, representing a 40% case fatality rate.[866] The majority of the infected are elderly males with one or more underlying medical conditions.[867] Persons infected with H7N9 often have direct exposure to infected birds at live poultry markets.[868] Family cluster analysis has suggested limited human-to-human transmission, but the restriction of transmission to within families hints at host-specific susceptibilities to H7N9 infection.[869] The R_0 of H7N9 has been consistently calculated below one. Specifically, the CDC calculated the R_0 to be 0.06 during the first wave of infections and 0.35 during the second wave, and other estimates have been similarly low.[870,871] Serological analysis of Chinese poultry workers revealed that 6% were seropositive for H7N9 infection but had experienced subclinical indications of infection.[872]

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859 Ostrowsky B *et al* (2012) Low pathogenic avian influenza A (H7N2) virus infection in immunocompromised adult, New York, USA, 2003. *Emerging infectious diseases* 18: 1128-1131

860 Abdelwhab EM *et al* (2014) Prevalence and control of H7 avian influenza viruses in birds and humans. *Epidemiol Infect* 142: 896-920

861 Tweed SA *et al* (2004) Human illness from avian influenza H7N3, British Columbia. *Emerging infectious diseases* 10: 2196-2199

Lopez-Martinez I *et al* (2013b) Highly pathogenic avian influenza A(H7N3) virus in poultry workers, Mexico, 2012. Ibid. 19: 1531-1534

862 Puzelli S *et al* (2005) Serological analysis of serum samples from humans exposed to avian H7 influenza viruses in Italy between 1999 and 2003. *J Infect Dis* 192: 1318-1322

863 Kurtz J *et al* (1996) Avian influenza virus isolated from a woman with conjunctivitis. *Lancet* 348: 901-902

864 Enserink M (2004) Infectious diseases. Bird flu infected 1000, Dutch researchers say. *Science (New York, NY)* 306: 590

865 Puzelli S *et al* (2014b) Human infection with highly pathogenic A(H7N7) avian influenza virus, Italy, 2013. *Emerging infectious diseases* 20: 1745-1749

866 FAO. H7N9 Situation Update. http://www.fao.org/ag/againfo/programmes/en/empres/H7N9/Situation_update.html. Last Update November 24, 2015. Accessed November 28, 2015.

867 Watanabe T *et al* (2014b) Pandemic potential of avian influenza A (H7N9) viruses. *Trends Microbiol* 22: 623-631

868 Li Q *et al* (2014a) Epidemiology of Human Infections with Avian Influenza A(H7N9) Virus in China. *New England Journal of Medicine* 370: 520-532

869 Jie Z *et al* (2013) Family outbreak of severe pneumonia induced by H7N9 infection. *Am J Respir Crit Care Med* 188: 114-115

Qi X *et al* (2013) Probable person to person transmission of novel avian influenza A (H7N9) virus in Eastern China, 2013: epidemiological investigation. *BMJ* 347: f4752

870 Kucharski AJ *et al* (2015) Transmission Potential of Influenza A(H7N9) Virus, China, 2013-2014. *Emerging infectious diseases* 21: 852-855

871 Chowell G *et al* (2013) Transmission potential of influenza A/H7N9, February to May 2013, China. *BMC Medicine* 11: 214

872 Yang S *et al* (2014) Avian-origin influenza A(H7N9) infection in influenza A(H7N9)-affected areas of China: a serological study. *J Infect Dis* 209: 265-269

Taken together, H7N2, H7N3, and H7N7 have demonstrated limited capacities to infect humans and have caused mild infections. In contrast, H7N9 has infected a large number of people over a short period of time, relative to other avian influenza viruses, and causes severe infection. Similar to H5N1, seroepidemiological studies suggest that many H7N9 infections are asymptomatic or sub-clinical, so that the "true" case fatality rate is likely lower than that estimated based on severe cases that interact with the healthcare system.

Avian influenza H9Nx strains

Since the first cases of human infection with avian influenza H9N2 in 1998 in Hong Kong, infections have been sporadically reported in humans and have caused relatively mild infections.[873,874,875,876,877] Epidemiological evidence suggests that H9N2 cannot transmit between people.[878] A systematic review of H9N2 seroprevalence in avian-exposed populations reported that between 1% and 43% of people had evidence of H9N2 infection, a high level of exposure that suggests that many infections are sub-clinical.[879] Taken together, these data demonstrate that H9N2 has a limited capacity to cause mild infections in humans and no current capacity for human-to-human transmission.

Avian influenza H10 strains

Two H10Nx strains have infected humans: H10N7 and H10N8. An avian H10N7 outbreak occurred in Australia during March of 2010. After culling, several abattoir workers displayed conjunctivitis and minor respiratory distress, and H10 infection was confirmed in two workers.[880] In December of 2013, an elderly woman died of H10N8 that she acquired from a LPM in the Nanchang, China.[881] Two subsequent cases of H10N8 were identified in Nanchang, and one patient died.[882] A serological analysis of H10N8 infection in LPM workers revealed that 21 had serological evidence of H10N8 infection despite no clinical indications of viral infection.[883] Taken together, these data demonstrate that H10Nx strains have limited capacity to infect humans but may cause severe disease, and that these strains have no current capacity for human-to-human transmission.

Reassortant strains

Human infections with reassortant strains containing avian H5, H7, or H9 genes, or the HA genes from other avian and swine viruses that have caused human infections (listed above), have not been recorded. However, all of the major influenza pandemics in the 20th and 21st centuries were caused by reassortant

873 Peiris M *et al* (1999a) Influenza A H9N2: aspects of laboratory diagnosis. *Journal of clinical microbiology* 37: 3426-3427
874 Peiris M *et al* (1999b) Human infection with influenza H9N2. *Lancet* 354: 916-917
875 Peiris M *et al* (1999c) Human infection with influenza H9N2. *Lancet* 354: 916-917
876 Butt KM *et al* (2005) Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. *Journal of clinical microbiology* 43: 5760-5767
877 "WHO | Antigenic and Genetic Characteristics of Zoonotic Influenza Viruses and Candidate Vaccine Viruses Developed for Potential Use in Human Vaccines," *WHO*, accessed August 26, 2015, http://www.who.int/influenza/vaccines/virus/characteristics_virus_vaccines/en/.
878 Uyeki TM *et al* (2002) Lack of evidence for human-to-human transmission of avian influenza A (H9N2) viruses in Hong Kong, China 1999. *Emerging infectious diseases* 8: 154-159
879 Khan SU *et al* (2015) A Systematic Review and Meta-Analysis of the Seroprevalence of Influenza A(H9N2) Infection Among Humans. *J Infect Dis* 212: 562-569
880 Arzey GG *et al* (2012) Influenza virus A (H10N7) in chickens and poultry abattoir workers, Australia. *Emerging infectious diseases* 18: 814-816
881 Chen H *et al* (2014) Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. *Lancet* 383: 714-721
882 Liu M *et al* (2015b) Genetic diversity of avian influenza A (H10N8) virus in live poultry markets and its association with human infections in China. *Sci Rep* 5: 7632
883 Qi W *et al* (2014a) Antibodies against H10N8 avian influenza virus among animal workers in Guangdong Province before November 30, 2013, when the first human H10N8 case was recognized. *BMC medicine* 12: 205

viruses that suddenly acquired the capacity for human to human transmission through antigenic shift. The 1918 H1N1 pandemic virus is thought to have arisen from reassortment between multiple avian strains, and all subsequent pandemic strains (1957, 1968, and 2009) are reassortants comprised of human seasonal and animal (avian and/or swine) gene segments.[884,885,886,887,888] Specifically, the 1957 H2N2 pandemic strain is a descendant of the 1918 H1N1 strain that acquired novel HA, NA, and PB1 genes from avian viruses, the 1968 H3N2 strain is a descendant of the 1957 H2N2 strain that acquired novel HA and PB1 genes from avian viruses, and the 2009 H1N1 strain is a triple reassortant strain comprised of genes of avian, swine, and human origin. Of note, the 1957 and 1968 pandemics were caused by HA subtypes that were not previously known to readily infect and transmit in humans. Thus, the historical record demonstrates that reassortment between human and animal viruses in nature can generate novel viruses with enhanced transmissibility in people, including viruses of HA subtypes not previously associated with human to human transmission. Of note, co-infection of people with H7N9 and either H3N2 or H1N1 has been detected, which could provide opportunities for the generation of reassortant viruses with enhanced transmissibility in people relative to the parental H7N9 strain.[889,890]

9.13.6.2 Laboratory Data – Characterization of Wild Type Viruses

Isolates of swine and avian influenza from human infections have been characterized for properties underlying mammalian adaptation and transmissibility, such as sialic acid receptor binding specificity, as well as infectivity and transmissibility in representative animal models. Similar to epidemiological data, these laboratory data speak to the current capacity for zoonotic influenza strains to infect and transmit in mammals. Additionally, if a given virus does not efficiently infect or transmit in representative animal models, the demonstration that it has acquired phenotypic properties thought to underlie mammalian adaptation and transmissibility (e.g., the ability to bind α2,6 sialic acid receptors) may speak to its potential to evolve the capacity for efficient infection and transmission of humans. That is, that virus may be poised to adapt to more efficiently infect and transmit in humans. This section reviews the phenotypic characteristics of wild type animal influenza strains isolated from human infections.

9.13.6.2.1 Swine Influenza H1N1v and H1N2v

No studies have evaluated the sialic acid receptor binding specificity or the transmissibility of H1N1v or H1N2v human isolates. Given that swine epithelial tissues express α2,6 sialylated receptors, it is likely that both are capable of binding to α2,6 receptors.[891]

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884 Smith GJD *et al* (2009c) Dating the emergence of pandemic influenza viruses. *PNAS* 106: 11709-11712

885 Antonovics J *et al* (2006) Molecular virology: was the 1918 flu avian in origin? *Nature* 440: E9;-discussion E9-10

886 Lu L *et al* (2014) Reassortment patterns of avian influenza virus internal segments among different subtypes. *BMC Evol Biol* 14: 16

887 Kawaoka Y *et al* (1989) Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. *Journal of virology* 63: 4603-4608
Scholtissek C *et al* (1978) Genetic relatedness between the new 1977 epidemic strains (H1N1) of influenza and human influenza strains isolated between 1947 and 1957 (H1N1). *Virology* 89: 613-617

888 Smith GJD *et al* (2009a) Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* 459: 1122-1125

889 Zhu Y *et al* (2013a) Human co-infection with novel avian influenza A H7N9 and influenza A H3N2 viruses in Jiangsu province, China. *Lancet* 381: 2134

890 Zhang W *et al* (2015) Co-infection with Avian (H7N9) and Pandemic (H1N1) 2009 Influenza Viruses, China. *Emerging infectious diseases* 21: 715-718

891 Trebbien R *et al* (2011) Distribution of sialic acid receptors and influenza A virus of avian and swine origin in experimentally infected pigs. *Virology Journal* 8: 434

9.13.6.2.2 Swine Influenza H3N2v

Clinical isolates of H3N2v were shown to exhibit a preference for binding to α2,6 sialylated receptors, to efficiently infect and transmit in ferrets by both contact and airborne routes of transmission, and to efficiently replicate in human cell lines. Taken together, those observations suggest that H3N2v viruses have the capacity for efficient replication and transmission in mammals.[892]

9.13.6.2.3 Avian Influenza H5Nx Strains

Wild type isolates of H5N1 infect but do not transmit via the airborne route between ferrets.[893] However, viruses isolated from patients infected with H5N1 have demonstrated binding capability to both avian-like α2,3 and human-like α2,6 receptors.[894] Several other strains of H5Nx that have not caused human infections have been evaluated for their virulence and transmissibility in ferrets as well as sialic acid receptor binding specificity. Similar to H5N1 isolates, an H5N5 strain isolated from poultry has been shown to bind both α2,3 and α2,6 sialic acids.[895] The North American H5N2 and H5N8 viruses that recently caused outbreaks in domestic poultry populations replicated efficiently in ferrets, but clinical symptoms were mild and neither virus was able to transmit in a direct contact setting.[896] A European H5N8 virus also exhibited low virulence in ferrets and was not transmitted via the respiratory route.[897]

9.13.6.2.4 Avian Influenza H7Nx Strains

Multiple H7Nx sub-types that have infected humans have demonstrated the capacity to bind α2,6 sialic acid receptors. Namely, an H7N2 virus isolated from poultry and patient isolates from the 2004 H7N3 outbreak in Canada exhibited enhanced affinity for α2,6 receptors, and H7N9 human isolates were capable of binding both α2,3 and α2,6 receptors.[898,899,900] Multiple H7Nx strains have also been shown to efficiently infect and transmit in ferrets. Human isolates from the Canadian H7N3 outbreak and an avian H7N7 isolate were contact transmissible between ferrets, and a recent H7N9 human isolate had the ability to transmit between ferrets via the airborne route.[900,902,903]

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892 Pearce MB *et al* (2012) Pathogenesis and transmission of swine origin A(H3N2)v influenza viruses in ferrets. *PNAS* 109: 3944-3949

893 Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

894 Shinya K *et al* (2005) Characterization of a Human H5N1 Influenza A Virus Isolated in 2003. *Journal of virology* 79: 9926-9932

895 Li Q *et al* (2015) Novel reassortant H5N5 viruses bind to a human-type receptor as a factor in pandemic risk. *Vet Microbiol* 175: 356-361

896 Pulit-Penaloza JA *et al* (2015) Pathogenesis and Transmission of Novel Highly Pathogenic Avian Influenza H5N2 and H5N8 Viruses in Ferrets and Mice. *Journal of virology* 89: 10286-10293

897 Richard M *et al* (2015) Low Virulence and Lack of Airborne Transmission of the Dutch Highly Pathogenic Avian Influenza Virus H5N8 in Ferrets. *PloS one* 10: e0129827

898 Belser JA *et al* (2008) Contemporary North American influenza H7 viruses possess human receptor specificity: Implications for virus transmissibility. *PNAS* 105: 7558-7563

899 Lopez-Martinez I *et al* (2013b) Highly pathogenic avian influenza A(H7N3) virus in poultry workers, Mexico, 2012. *Emerging infectious diseases* 19: 1531-1534
Tweed SA *et al* (2004) Human illness from avian influenza H7N3, British Columbia. Ibid. 10: 2196-2199

900 Ramos I *et al* (2013a) H7N9 influenza viruses interact preferentially with α2,3-linked sialic acids and bind weakly to α2,6-linked sialic acids. *J Gen Virol* 94: 2417-2423
Xiong X *et al* (2013) Receptor binding by an H7N9 influenza virus from humans. *Nature* 499: 496-499

901 Belser JA *et al* (2008) Contemporary North American influenza H7 viruses possess human receptor specificity: Implications for virus transmissibility. *PNAS* 105: 7558-7563

902 Belser JA *et al* (2014) Influenza virus infectivity and virulence following ocular-only aerosol inoculation of ferrets. *Journal of virology* 88: 9647-9654

903 Zhang Q *et al* (2013b) H7N9 influenza viruses are transmissible in ferrets by respiratory droplet. *Science (New York, NY)* 341: 410-414

9.13.6.2.5 Avian Influenza H9N2 Strains

Characterization of H9N2 strains isolated from poultry in live poultry markets in China between 2009 and 2013 found that several exhibited a preference for binding to α2,6 sialic acid receptors (though retained the ability to bind α2,3 receptor) and were capable of airborne transmission between ferrets.[904]

9.13.6.2.6 Avian Influenza H10Nx Strains

H10N8 viruses isolated from ducks have exhibited broad sialic acid receptor binding capabilities, to both α2,3 and α2,6 receptors.[905] H10N7 isolates from human and avian sources have also demonstrated broad sialic acid receptor binding specificity.[906] Wild type isolates of neither strain have been characterized for transmissibility.

9.13.6.3 Computational Modeling Data

Computational models for virus evolution can be used to explore the likelihood that a given set of mutations shown to confer enhanced transmissibility in a laboratory setting will evolve in nature. For example, following the identification of sets of mutations that were sufficient to confer airborne transmissibility to H5N1 viruses by the Kawaoka and Fouchier research groups, another group evaluated the likelihood that currently circulating H5N1 strains could evolve those mutations during passage through a single human host.[907] The authors consider several different evolutionary contexts including various selection pressures, the need to acquire a different number of mutations (based on the number of mutations in the starting virus), and varying lengths of infection time. The authors conclude that it is possible for H5N1 to evolve the set of mutations shown to confer the capacity for respiratory droplet transmission within a mammalian host, supporting the idea that the evolutionary pathway identified in the laboratory studies is possible in nature.

Another research group used a modeling approach to predict the length of time needed for the H7 protein from H7N9 viruses that have infected humans to acquire mutations that would render it structurally and genetically similar to H3 proteins from human seasonal H3N2 viruses. Their model estimated that this evolution, which may result in H7N9 viruses that are human to human transmissible, requires approximately eleven years.[908]

Notably, the results of these and other evolutionary modeling studies are subject to significant uncertainty due to uncertainties in the values of the parameters used to build the models, among other factors.

9.13.6.4 Conclusions

Laboratory experiments have enhanced the transmissibility of animal influenza strains that do not efficiently transmit between humans in nature through the direct evolution pathway (i.e., the incorporation

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904 Li X *et al* (2014b) Genetics, receptor binding property, and transmissibility in mammals of naturally isolated H9N2 Avian Influenza viruses. *PLoS Pathog* 10: e1004508
Matrosovich MN *et al* (2001) H9N2 Influenza A Viruses from Poultry in Asia Have Human Virus-like Receptor Specificity. *Virology* 281: 156-162

905 Deng G *et al* (2015) Genetics, Receptor Binding, and Virulence in Mice of H10N8 Influenza Viruses Isolated from Ducks and Chickens in Live Poultry Markets in China. *Journal of virology* 89: 6506-6510

906 Ramos I *et al* (2015) Hemagglutinin Receptor Binding of a Human Isolate of Influenza A(H10N8) Virus. *Emerging infectious diseases* 21: 1197-1201

907 Russell CA *et al* (2012) The Potential for Respiratory Droplet–Transmissible A/H5N1 Influenza Virus to Evolve in a Mammalian Host. *Science* 336: 1541-1547

908 Peng J *et al* (2014) The origin of novel avian influenza A (H7N9) and mutation dynamics for its human-to-human transmissible capacity. *PloS one* 9: e93094

of mutations through serial passaging or targeted mutagenesis) and/or through reassortment with seasonal influenza viruses. To shed light on whether these laboratory-generated phenotypic changes could occur in nature, three types of data were reviewed: epidemiological data about the number and patterns of human infections with animal influenza viruses, laboratory data about the phenotypic characteristics of animal influenza viruses isolated from human infections, and computational modeling data about the evolutionary capacity of these viruses. The findings are summarized and synthesized below.

Avian and swine influenza viruses currently exhibit limited capacity to infect and transmit in humans, though H5N1 and H7N9 viruses are capable of causing severe disease in the event of a human infection.[909,910,911,912] Human infections with reassortant viruses containing gene segments from avian or swine viruses that have infected humans have not been observed, but co-infections of people with avian and human seasonal viruses have been reported, which could provide opportunities for the emergence of novel reassortant viruses with enhanced transmissibility in humans. Laboratory characterization of human isolates of avian and swine flu viruses have shown that some H3N2v and H7N9 viruses are capable of airborne transmission between ferrets. Other sub-types (including H5N1 and H9N2) do not transmit in representative animal models, but human isolates of these viruses have the ability to bind "human-like" α2,6 sialic acid receptors, thought to be critical for efficient infection and transmission in humans. Collectively, these phenotypic data suggest that these viruses may have partially evolved the capacity for human to human transmission. Finally, computational modeling suggests that the set of adaptive mutations needed to confer the capacity for airborne transmission in mammals to H5N1 viruses can accrue during a single round of transmission in a human host.

Taken together, the evolutionary implications of these observations – i.e., that some animal flu subtypes (H3N2v, H5N1, H7N9, and H9N2) continue to infect humans and share some of the phenotypic characteristics of viruses that do efficiently infect and transmit in humans – are uncertain. On the one hand, fully avian or swine viruses are not known to have directly evolved the capacity for efficient transmission in humans. Some have argued that the large number of human infections with these viruses, including the many mild or sub-clinical infections that are indicated by seroepidemiology studies, have provided ample opportunities for transmissibility to evolve if that were possible. In particular, avian influenza H5N1 strains first caused human infections over 15 years ago, in 1997.[913] On the other hand, the historical record, comprising just four influenza pandemics, represents a scant source of data from which to draw conclusions about what evolutionary pathways are or are not possible, as well as the length of time that is or is not "sufficient" for a particular evolutionary change to occur. Moreover, the historical record shows that influenza pandemics have occurred on average every 25 years, with an interim pandemic period of up to forty years (i.e., 1918 and 1957 pandemics) – longer than the length of time that H5N1 strains have been sporadically infecting people. In addition, socio-cultural factors that critically influence the evolution of influenza viruses in human populations, in particular the nature of human interactions with animals and the environment, change over time. These changes will further compromise the relevance of predictions about viral evolution based on historical data. Critically, the fact that fully avian influenza strains have adapted to efficiently transmit between dogs definitively demonstrates that cross-species adaptation of avian viruses to mammals is possible.

What is clear from the historical record is that enhanced transmissibility in humans can arise through reassortment between human seasonal and animal influenza strains, including the generation of viruses of

[909] Gao R *et al* (2013) Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med* 368: 1888-1897
[910] Watanabe T *et al* (2013) Characterization of H7N9 influenza A viruses isolated from humans. *Nature* 501: 551-555
[911] Hatta M *et al* (2001) Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science* 293: 1840-1842
[912] Katz JM *et al* (2000) Molecular correlates of influenza A H5N1 virus pathogenesis in mice. *Journal of virology* 74: 10807-10810
[913] WHO. Avian Influenza Fact Sheet. http://www.who.int/mediacentre/factsheets/avian_influenza/en/. Last Update September 2014. Accessed November 28, 2015.

HA subtypes that are "novel" to the human population (e.g., the 1957 H2N2 pandemic virus and the 1968 H3N2 pandemic virus). Importantly, lessons learned from laboratory studies focusing on fully avian or swine strains, which explore pathways for directly evolving enhanced transmissibility, may be generalizable to both wholly avian/swine influenza strains and mixed-species reassortment strains. For example, both H5N1 transmissibility studies published in 2012 uncovered the same HA stability phenotype underlying airborne transmissibility in ferrets, despite the fact that one study involved an HPAI H5N1 strain whereas another involved a 7:1 reassortant with a seasonal H1N1 strain. Thus, even if avian or swine strains are unlikely to directly evolve to efficiently transmit in humans in nature, transmission studies involving fully avian or swine strains may provide information that is relevant to the behavior of reassortment strains.

9.14 Evaluation of the Globalization Potential of GoF Research

9.14.1 Summary of Findings

Whether risks and benefits are equally distributed across populations is an important consideration in any risk-benefit comparison. For GoF research involving PPPs, the risks are global. This section provides an overview of the potential for select benefits of GoF research conducted in the US to diffuse globally, in order to inform the comparison of risks and benefits associated with this research. A fully referenced and more thorough discussion of these benefits can be found in Appendix IV Section 15.9.

The potential for three types of GoF benefits to globalize are considered:

- Improvements to the production of egg- and cell-based influenza vaccines,
- Assistance in the development of new influenza and coronavirus small molecule antivirals, and
- Contributions to risk assessments of circulating animal influenza viruses (pre-pandemic), which in turn inform prioritization of pandemic preparedness activities such as the development of pre-pandemic vaccines.

9.14.1.1 Improvements to the Production of Egg- and Cell-Based Influenza Vaccines

Several developing countries have the capacity to directly harness GoF research that benefits the production of egg- and cell-based influenza vaccines. Specifically, non-high income countries host 18 vaccine producers spanning eight countries, representing an increase in the number of producers and vaccine-producing countries since 2010. However, the establishment of new influenza vaccine production lines in foreign countries is a slow process – on the order of eight years or longer – and is hampered by political, technical, and economic factors. Lack of demand for influenza vaccines in-country appears to be a particularly important issue facing all producers, which is compounded by a lack of knowledge about optimal vaccination strategies in tropical regions.

US vaccine donations in the event of a pandemic provide a second pathway for GoF-derived benefits to reach developing countries. The United States donated approximately 14% of the vaccines committed to the WHO during the 2009 H1N1 pandemic response, which collectively were deployed to 77 countries. However, in 2009 both vaccine donation and distribution were significantly delayed, and logistical challenges associated with vaccine distribution further reduced and/or delayed the quantity of vaccine doses that reached developing countries' populations. Although some of these shortcomings have been addressed in theory by the WHO Pandemic Influenza Preparedness Framework, the ability of the US and

the WHO to provide donated vaccines in time to mitigate the effects of a high morbidity influenza pandemic in the world's developing countries remains untested.

9.14.1.2 Assistance in the Development of Novel Influenza or Coronavirus Antivirals

The ability of foreign countries to establish production lines for new antivirals depends not only on their technical and industrial capabilities but also on their ability to negotiate complex patent issues. In cases where patent protections do not apply, the actual time needed to initiate commercial production of a US-designed or commercialized antiviral appears to be in the one to five year range. However, several companies in developing countries rapidly activated production of influenza antivirals in less than six months in 2005– 2006, when their governments were preparing for a potential H5N1 pandemic, suggesting that a general lack of demand for influenza antivirals appears to be keeping globalization in check.

The US demonstrated its willingness to donate influenza antivirals during the 2009 H1N1 pandemic. However, problems of timeliness of supply compounded issues of suboptimal use in-country. The WHO Pandemic Influenza Preparedness Framework (developed in 2011) seeks to address timeliness issues but remains untested.

9.14.1.3 Contributions to Pandemic Risk Assessments of Circulating Influenza Viruses

The demonstration that animal influenza viruses can acquire pandemic properties in a laboratory setting may galvanize preparedness efforts in developing countries where the virus is circulating in agricultural animal or wildlife populations.

Because most developing countries in which high-risk animal influenza viruses are circulating lack the ability to assess the transmissibility and virulence of viruses in ferrets, data which critically inform pandemic risk assessments, risk assessments are carried out in collaboration with the WHO and laboratory members of the GISRS (including the CDC). Similar to USG risk assessments, these risk assessments incorporate information derived from GoF research, alongside epidemiologic and virologic data, and environmental factors that influence the pandemic potential of the virus.

Downstream of a pandemic risk assessment, the ability of developing countries to implement prevention and early detection measures in response to the detection of zoonotic influenza cases or outbreaks in humans and/or animals varies widely, depending on the state of public health infrastructure, the relationship between the Veterinary Services and Public Health sectors, and the resources for investing the prevention and response activities. Although multiple developing countries in which zoonotic avian influenza infections have been detected in human and/or bird populations within the past five years currently have the capacity to produce pre-pandemic influenza vaccines in-country, 21 do not. As the WHO does not stockpile pre-pandemic vaccines, the lack of vaccine production capabilities in some at-risk countries limits the globalization potential of GoF benefits related to pandemic risk assessments.

9.14.2 Introduction

Whether risks and benefits are equally distributed across populations is an important consideration in any risk-benefit comparison. For GoF research involving PPPs, the risks– that biosafety or biosecurity incidents associated with the conduct of GoF research involving PPPs may spark a pandemic–are global. To inform the assessment of global risks versus benefits, this section evaluates the globalization potential of select GoF benefits. Specifically, the potential for the outputs of GoF research conducted in the US to benefit the health of human populations in low- and middle-income bracket countries, as defined by the

World Bank, is analyzed.[914]

Three types of GoF benefits are considered in this section:

- Benefits to the development and production of egg- and cell-based influenza vaccines,
- Benefits to the development of new antivirals for influenza viruses or coronaviruses, and
- Benefits to risk assessments of circulating animal influenza viruses (pre-pandemic), which may in turn stimulate pandemic preparedness activities such as enhanced surveillance and the development of pre-pandemic vaccines.

Currently, there are no FDA-approved vaccines for MERS-CoV or SARS-CoV.[915,916] GoF research involving CoV has potential to benefit the development of CoV vaccines, which is an active area of research involving a variety of vaccine platforms. Which type of vaccine will prove to be most effective is not yet clear based on current research. Because the resources and expertise that are required to develop production capacity for different types of vaccines varies, the globalization potential and barriers to globalization for hypothetical CoV vaccines cannot be evaluated. Similar uncertainties preclude evaluation of GoF benefits to the development of new influenza vaccines. For these reasons, the assessment of GoF benefits to vaccines is limited to those benefits to the development and production of existing influenza vaccines.

The globalization potential of GoF benefits to therapeutics is evaluated based on case studies of the four influenza antivirals that are currently licensed in developed countries, each of which is a small molecule compound initially developed in a high-income country. This assessment assumes that setting up hypothetical future production lines for new small molecule drugs targeting CoVs or influenza will require a similar level of resources as was needed to set up production lines for existing influenza antivirals. As a result, the conclusions herein about the globalization potential of GoF benefits to therapeutics apply to GoF research involving both influenza viruses and CoVs that may inform the development of new small molecule drugs.

As GoF research involving CoVs does not currently benefit surveillance or decision-making in public health policy, the assessment of the globalization potential of GoF benefits to pandemic risk assessments is limited to research involving influenza viruses.

Below, the globalization potential of each of the three GoF benefits list above is evaluated in turn.

9.14.3 Potential Benefit 1- Improvements in the Design and Production of Vaccines

Several types of GoF research have potential to improve the development and production of egg- and cell-based influenza vaccines, namely GoF research that enhances virus production, leads to evasion of therapeutics, enhances pathogenicity, and leads to evasion of existing natural or induced adaptive immunity. In brief, GoF research that enhances virus production leads to the generation of higher-yield

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914 This classification system is used by the World Health Organization.
The World Bank, "Country and Lending Groups," http://data.worldbank.org/about/country-and-lending-groups. Accessed July 7, 2015.

915 Centers for Disease Control and Prevention (CDC), "Middle East Respiratory Syndrome (MERS)," June 2, 2015, http://www.cdc.gov/coronavirus/mers/about/prevention.html. Accessed July 7, 2015.

916 World Health Organization, "Severe Acute Respiratory Syndrome (SARS)," December 1, 2013, http://www.who.int/immunization/topics/sars/en/. Accessed July 7, 2015.

vaccine viruses, which can improve the availability of pandemic flu vaccines and the efficacy of seasonal flu vaccines by shortening vaccine production timelines. Increasing the yield of vaccine antigen per egg or cell also reduces the manufacturing cost of the vaccine, which may translate to a lower cost per vaccine dose. GoF research that enhances virulence and leads to evasion of therapeutics may lead to the identification of molecular markers for virulence and antiviral resistance, respectively, that can be removed from vaccine viruses through targeted mutagenesis, thereby increasing the safety of the vaccine production process. Finally, GoF research that leads to the evasion of existing natural or induced immunity has potential to improve the strain selection process for seasonal flu vaccines, thereby increasing their efficacy. Each of these benefits may be harnessed by developing countries through direct application of GoF research outputs to indigenous influenza production lines, or may benefit developing countries indirectly through US seasonal and pandemic vaccine donations.

9.14.3.1 Capacity for Direct Application of GoF Research Outputs to Foreign Influenza Vaccine Production

High yield candidate vaccine viruses (CVVs) for seasonal and pandemic influenza strains, which serve as the basis for vaccine strains used for large-scale manufacturing of vaccines, are developed by WHO Collaborating Centres (WHOCCs) for Influenza and other collaborating laboratories.[917,918,919] The WHO Pandemic Influenza Preparedness Framework stipulates that influenza CVVs be made available from WHOCCs to any influenza vaccine manufacturer and any other laboratory who makes a request, as long as the requestor meets appropriate biosafety requirements to receive the strain in question.[920] The GISRS provides the international framework for the sharing of such biological materials between laboratories around the world.[921] Thus, any GoF benefits to strain selection for seasonal flu vaccines (which determines the composition of CVVs) are inherently global. Other GoF benefits to influenza vaccine production, which involve the discovery of molecular markers for high yield, virulence, and antiviral resistance, can be incorporated into vaccine viruses by CVV developers or vaccine manufacturers. Therefore, developing countries with industrial capacity to produce influenza vaccines have the ability to directly benefit from GoF research conducted in the US, through utilization of modified CVVs provided by WHOCCs or through the application of GoF research findings to vaccine strains developed by indigenous manufacturers. Altogether, the likelihood and timescale over which GoF benefits to vaccine production can be realized depends on two factors: (1) for those countries that do not yet have influenza vaccine production capabilities, the resources needed for the establishment of new influenza vaccine production lines and (2) for those countries that already have influenza vaccine production capabilities, the country's regulatory policies governing changes in vaccine strains. Although an assessment of country-specific regulatory policies as they pertain to the use of modified vaccine strains is outside the scope of the current study, the FDA does not require regulatory approval for the commercial use of modified vaccine strains (i.e., there is no regulatory barrier for GoF benefits to vaccine production in the US).

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917 World Health Organization (WHO), "Influenza: Influenza vaccine viruses and reagents," http://www.who.int/influenza/vaccines/virus/en/. Accessed July 7, 2015.

918 World Health Organization (WHO), "Influenza: Virus Sharing," http://www.who.int/influenza/pip/virus_sharing/en/. Accessed July 7, 2015.

919 World Health Organization (WHO), *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits* (Geneva: World Health Organization Press, 2011), p. 16-17, http://apps.who.int/iris/bitstream/10665/44796/1/9789241503082_eng.pdf. Accessed July 7, 2015.

920 Ibid.

921 World Health Organization (WHO), "Global Health Observatory (GHO) data: Global influenza virological surveillance," http://www.who.int/gho/epidemic_diseases/influenza/virological_surveillance/en/. Accessed July 7, 2015.

9.14.3.2 Capacity for Direct Application of GoF Research Outputs to Foreign Influenza Vaccine Production

Global influenza production capacity was most recently comprehensively surveyed in 2010 by the WHO. The WHO study identified 14 manufacturers in middle-income countries, collectively marketing at least eleven vaccines and developing at least another eight vaccines. [922,923] No updated list of active human influenza vaccine manufacturers in 2014 or 2015 has been made publicly available. A dataset of current influenza producers was therefore compiled to compare the current influenza production situation with that surveyed in 2010.[924] The results are summarized in the figure below, and a reference list is provided in Section 16.9.6.

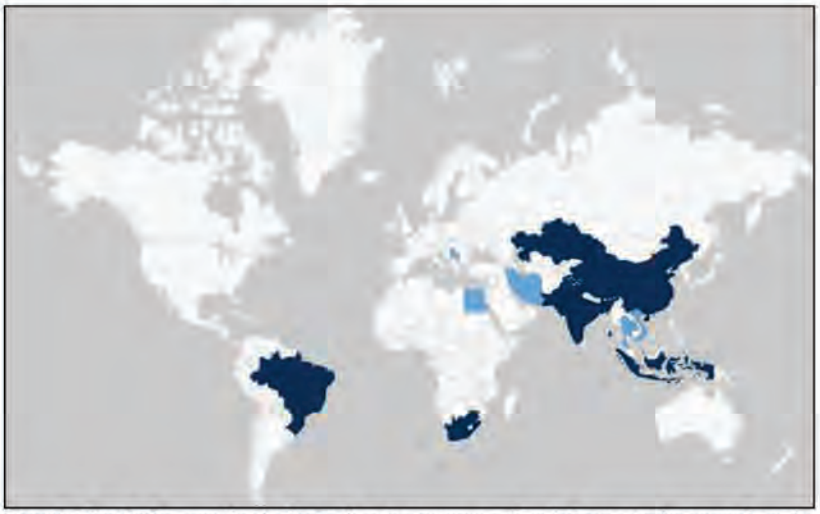

Figure 9.6. Developing countries that host at least one company with an influenza vaccine currently on the market are shaded in deep blue. Developing countries that host at least one company with R&D efforts for the production of an influenza vaccine are shaded in light blue.

Analysis of the assembled dataset reveals that the number of active producers outside of high-income countries has increased since 2010. In total, 18 companies in middle-income countries were found to be actively producing influenza vaccines, and at least 13 additional companies have R&D work for influenza vaccines at various stages of completion, compared to 14 manufacturers with current or planned flu

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922 WHO. Technical studies under resolution WHA63.1. Final Document. http://apps.who.int/gb/pip/pdf_files/OEWG3/A_PIP_OEWG_3_2-en.pdf. Last Update April 4, 2011. Accessed January 26, 2016.

923 The survey identified the following five middle-income countries as having domestic influenza vaccines: China, India, Thailand, Indonesia, and Romania. Planned production lines were identified in the following nine middle-income countries: Brazil, Egypt, Kazakhstan, Mexico, Serbia, South Africa, Thailand, Iran, and Vietnam.

924 This dataset was compiled from lists of vaccine manufacturers in the 2010 WHO survey, the Developing Countries Vaccine Manufacturers Network (DCVMN) directories from 2014 and 2015, the International Federation of Pharmaceutical Manufacturers & Associations' Influenza Vaccine Supply Members list, and the U.S. Department of Health and Human Services' Influenza Vaccine International Capacity Building Portfolio, supplemented by searches of additional manufacturers mentioned in the literature or in news reports.

vaccine production lines in 2010.[925] However, as many of the new influenza vaccine manufacturers since 2010 are located in countries that already had influenza vaccine production capabilities, overall the geographic distribution of production capacities outside of high-income countries has only moderately expanded. Eight countries now produce influenza vaccines (up from five). Based on current R&D efforts, an additional five countries may become influenza vaccine producers in the future.[926]

A lack of end-user demand appears to be a recurring and common problem that is preventing several of the middle-income firms mentioned in this section from initiating or maintaining influenza vaccine production. With respect to pandemic influenza vaccines, this issue stems from a lack of government support to purchase vaccines for pandemic preparedness purposes. With respect to seasonal influenza vaccines, this issue involves a lack of demand by individuals. Notably, the Chinese market experience has demonstrated that domestic demand for seasonal influenza vaccine increases with the income level of individuals, thus low domestic demand is to be expected outside of high income countries.[927] This demand issue is compounded by the fact that current recommendations for the strain composition of seasonal influenza vaccines are geared toward countries in the Northern and Southern hemispheres with well-defined flu seasons, such as the United States and Australia.[928] In contrast, well-defined seasonality does not always occur in tropical regions of the world; instead, low levels of influenza virus circulate throughout the year. In these regions, optimal vaccination strategies, including whether Northern or Southern hemisphere vaccines are more protective and when during the year vaccines are best deployed, are not well understood. Research to better understand patterns of influenza transmission and seasonality in the tropics, as well as how best to mitigate the public health burden associated with influenza through vaccination, is ongoing. This research provides an important foundation for developing countries' efforts to bolster their vaccine production capabilities and increase in-country demand in the future.

Several US programs seek to support the aforementioned ability of developing countries to produce vaccines. Since seasonal vaccine production lines are adapted to produce pandemic vaccines, these pandemic preparedness programs complement seasonal influenza production assistance, and vice versa.[929]

The US HHS supports production capabilities abroad for seasonal and pandemic influenza vaccine through funding provided by its Biomedical Advance Research and Development Authority (BARDA) branch.[930] Overall, BARDA has provided over $70 million in financial support to 13 companies in 12

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925 This count excludes companies based in Taiwan, as the World Bank classes "Taiwan, China" as a "high-income" economy, separately from "China," which it classes as an "upper-middle-income" economy. See:
The World Bank, "Country and Lending Groups," http://data.worldbank.org/about/country-and-lending-groups. Accessed July 7, 2015.

926 Namely: Egypt, Iran, Serbia, Thailand, and Vietnam.

927 Eliza Yibing Zhou, "Vaccine Development in China," *BioPharm International* 20, no. 4 (April 2007): p.1, http://www.biopharminternational.com/china-today-vaccine-development-china. Accessed October 29, 2015.

928 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. *Vaccine* 33: 4368-4382

929 For U.S. context, see: Executive Office of the President, President's Council of Advisors on Science and Technology, [U.S.A.] "Report to the President on Reengineering the Influenza Vaccine Production Enterprise to Meet the Challenges of Pandemic Influenza," August 2010, https://www.whitehouse.gov/sites/default/files/microsites/ostp/PCAST-Influenza-Vaccinology-Report.pdf. Accessed July 7, 2015.

930 PATH, "PATH's Work in Vaccine Development: Low-cost influenza vaccine production," http://sites.path.org/vaccinedevelopment/influenza/vaccine-production-in-the-developing-world/. Accessed August 3, 2015.

middle-income countries seeking to develop influenza vaccine production lines since 2006.[931, 932, 933] Of the 13 companies that received support from BARDA, six appear to remain in the R&D phase, one has ceased production of vaccines, one appears to have halted R&D efforts, and five currently produce influenza vaccines. Impediments to the establishment of production lines include human factors (e.g., alleged corruption delaying construction of manufacturing facilities), technical factors (e.g., contamination of vaccine doses), and economic factors (e.g., lack of domestic demand). (For additional details, see Table 16.40 in Section 16.9.3). Thus, more than eight years after BARDA began its assistance program, roughly two thirds of the funding recipients appear to lack an influenza vaccine product on the market. The four successful companies demonstrate that *some* developing countries are able to develop, produce, and market a new influenza vaccine given eight years. However, the human, technical, and economic problems encountered by the other companies drive home the point that setting up new influenza vaccine production lines is time-consuming and is a high-risk endeavor from a business perspective.

9.14.3.3 Capacity of GoF benefits to Vaccine Production to Globalize Through US Vaccine Donations

The United States supports foreign seasonal and pandemic influenza vaccine stockpiles through direct vaccine donations, which represents a different pathway for the globalization of GoF benefits related to vaccine development and production. Specifically, any GoF-derived improvements to US vaccine development and production will indirectly benefit developed countries that receive US-produced vaccines through assistance and emergency response programs.

9.14.3.3.1 US Seasonal Vaccine Donations

The US Department of Health & Human Services's Centers for Disease Control has recently begun donating seasonal vaccines in an effort to increase seasonal influenza vaccination in developing countries. The US CDC organizes the donation of seasonal influenza vaccines as part of the vaccine donation portion of the Partnership for Influenza Vaccine Introduction.[934] Since 2012, domestic companies involved in the production, distribution, and sales of seasonal influenza vaccines have donated up to 375,000 doses of seasonal vaccine annually to developing countries.[935,936,937] However, several factors significantly limit the impact of this program. First, donations are "based on [the] availability of excess vaccine supply" and are therefore unpredictable and potentially limited.[938] Second, the WHO guidelines stipulate that the vaccine must be licensed for use in the recipient country, which excludes many countries without domestic influenza vaccine production capabilities and relevant regulatory infrastructure.[939]

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931 These companies are: Accra de Birmex (Mexico), BCHT (China), BioFarma (Indonesia), Cantacuzino Institute (Romania), GPO (Thailand), Instituto Butantan (Brazil), IVAC (Vietnam), RIBSP (Kazakhstan), Serum Institute of India (India), The BioVac Institute (South Africa), Torlak Institute (Serbia), VABIOTECH (Vietnam), and VACSERA (Egypt).

932 U.S. Department of Health and Human Services. International Influenza Vaccine Capacity Building Portfolio. https://www.medicalcountermeasures.gov/projectmaps/Who.aspx. Last Update Accessed January 26, 2016.

933 United States of America, "Report on USA implementation of Article X of the Biological and Toxin Weapons Convention," Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, Meeting of Experts, Geneva, Switzerland, August 4-8, 2014, BWC/MSP/2014/MX/INF.5, p.4 para. 10. Accessed July 7, 2015.

934 The Task Force for Global Health, "Partnership for Influenza Vaccine Introduction," <http://www.taskforce.org/our-work/projects/partnership-influenza-vaccine-introduction>.

935 Joseph Bresee, CDC, "Global Action Plan for Influenza Vaccines – II: CDC's Supportive Activities," GAP-II Partners Meeting, Dubai, United Arab Emirates, March 18, 2013, <http://www.who.int/phi/Day1_9_Bresee_GAP2_CDC_PM_Dubai2013.pdf>.

936 Alan R. Hinman, "Partnership for Influenza Vaccine Introduction (PIVI)," Dubai, United Arab Emirates, March 25, 2014, p.2. <http://www.who.int/phi/DAY1_08_Panel2_Hinman_Panel2_PIVI_PM_Dubai2014.pdf>.

937 Centers for Disease Control and Prevention (CDC), "Laos and Nicaragua Protect High-Risk Persons from Influenza, with Help from Donor Coalition and CDC," <http://www.cdc.gov/flu/international/highlight-high-risk.htm>.

938 Alan R. Hinman, "Partnership for Influenza Vaccine Introduction (PIVI)," p. 5.

939 Ibid.

Finally, the timing of US seasonal vaccine donations may not match the recipient country's influenza season, further limiting the number of countries that may benefit from the donated vaccine doses.[940] Taken together, these limitations significantly constrain the number of countries that can receive US donations under these programs.

9.14.3.3.2 US Vaccine Donations in Response to a Pandemic

In the event of a pandemic, US national policy calls for donations of vaccines to the WHO for redistribution to developing countries. As a member state to the WHO Pandemic Influenza Preparedness Framework, the US is committed to supplying influenza vaccines to a WHO-maintained pandemic benefit-sharing system, which would then redistribute vaccines to developing countries as necessary to respond to a pandemic.[941] Although the exact quantity to be contributed by each member state is not specified, the document makes clear that the vaccine donations should be structured as a percentage of vaccine production runs, to ensure timely supply.[942]

The following case study on the US vaccine donations in response to the 2009 H1N1 pandemic show how and to what extent US vaccine donations can reach developing countries. The 2009 pandemic preceded and motivated the formation of the WHO's Pandemic Influenza Preparedness Framework in 2011. As such, although the actions taken by the US during the pandemic remain instructive, certain shortcomings in the international donation and response system have been addressed by the establishment of a Framework.

During the H1N1 influenza pandemic, US vaccine donations were organized in response to 17 bilateral requests and a call for "global solidarity" from the WHO Director General.[943] In September 2009, the United States pledged up to 10% of its vaccine production runs to the WHO; eight other countries subsequently made similar pledges.[944] The US H1N1 influenza response established a "10%" rule of thumb, whereby 10% of vaccine production runs would be donated to the WHO for distribution to developing countries in need of assistance. In total, the United States donated 16,860,100 doses of 2009 H1N1 influenza vaccine to the WHO for international distribution, which represented approximately 14% of the vaccines committed to the WHO.[945,946] Out of a total of 122,450,000 vaccine doses committed by all states, the WHO distributed a total of 78,066,290 doses of vaccines to 77 countries.[947]

Overall, donation of vaccines to WHO suffered from severe timeliness issues. Vaccine production and domestic supply difficulties in the US (and other developed countries) in turn delayed vaccine donations

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940 Ibid.

941 World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits* (Geneva: World Health Organization Press, 2011), p. 15-16, 18.

942 Ibid.

943 "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," p. 86, http://www.phe.gov/Preparedness/mcm/h1n1-retrospective/Documents/h1n1-retrospective.pdf.

944 The eight countries were: Australia, Brazil, France, Italy, New Zealand, Norway, Switzerland, and the United Kingdom. World Health Organization, "Report of the WHO Pandemic Influenza A(H1N1) Vaccine Deployment Initiative," 2012, p. 4, http://www.who.int/influenza_vaccines_plan/resources/h1n1_deployment_report.pdf.

945 United States of America, "Identifying and addressing barriers to the emergency sharing of international public health and medical assistance," Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, Meeting of Experts, Geneva, Switzerland, August 12-16, 2013, BWC/MSP/2013/MX/WP.6, p. 2 para. 5.

946 "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," p. 87, http://www.phe.gov/Preparedness/mcm/h1n1-retrospective/Documents/h1n1-retrospective.pdf.

947 The commitment of vaccines to the WHO involves a signed agreement, and therefore goes beyond a political pledge. World Health Organization, "Final Pandemic (H1N1) 2009 Vaccine Deployment Update," November 10, 2010, http://www.who.int/csr/disease/swineflu/action/h1n1_vaccine_deployment_final_update_2010_11_10.pdf.

to the WHO.[948,949] Advanced purchase agreements, whereby a given number of vaccines not yet produced are purchased by a government from a private vaccine producer, compounded accessibility issues.[950] Since the vaccines already belonged to a particular buyer, the private firm was unable to donate a portion of the run to the WHO, regardless of a desire to do so.[951] Other developed countries were reticent in donating vaccines, and in a particularly severe pandemic, whether promised doses would reach developing countries in time to be effective is unclear.[952] Several developed countries– such as France, Germany, Switzerland, and the Netherlands– tried to sell excess vaccines instead of donating them.[953,954] The WHO Pandemic Influenza Preparedness Framework's explicit clause on the provision of vaccines on a rolling basis seeks to prevent this particular donation timeliness problem, but whether countries will comply with the Framework during a severe pandemic remains untested.[955] In addition to delays in the donation of vaccine doses, the planning and execution of the donation and distribution of vaccine doses and ancillary supplies was hampered by several logistical, regulatory, and political factors that further delayed and/or reduced the quantity of vaccine doses distributed to recipient countries.

Taken together, these challenges highlight that while US donation of vaccines is a viable pathway by which GoF benefits to vaccine production may globalize, the time needed to orchestrate the logistics of vaccine shipment and vaccination in-country will delay delivery of a vaccine to a developing country's population relative to a scenario in which that country is capable of indigenously producing and freely distributing its own vaccine doses.

9.14.3.4 Summary – Globalization Potential of GoF Benefits to Influenza Vaccine Production

GoF benefits to the production of influenza vaccines can be realized by developing countries in two ways: (1) through the direct application of GoF research insights to production in-country and (2) through the receipt of US-produced vaccines donated through assistance or emergency response programs.

With respect to indigenous production capabilities, both the total number of vaccine *producers* outside of high-income countries (17) and the number of non-high income producing *countries* (7) has increased since 2010. As WHOCCs provide ready access to candidate vaccine strains to all such producers, these countries are currently capable of harnessing GoF research benefits to vaccine production. The total number of producers outside of high-income countries is slated to increase by as many as an additional six countries in the near future given current R&D efforts by over a dozen companies spanning eight different countries. Analysis of the R&D timelines for foreign influenza vaccine manufacturers that received BARDA funding support shows that bringing a new influenza vaccine to market may require up to eight years, and that many efforts to develop new production lines fail due to political, technical, and

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948 David P. Fidler, Kelly Lee, "Negotiating Equitable Access to Influenza Vaccines: Global Health Diplomacy and the Controversies Surrounding Avian Influenza H5N1 and Pandemic Influenza H1N1," *PLoS Med* 7, no. 5 (May 2010), http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2864298/.

949 Supriya Kumar et al., "US Public Support for Vaccine Donation to Poorer Countries in the 2009 H1N1 Pandemic," *PLoS One* 7, no. 3 (2012), http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3295778/.

950 Sam F. Halabi "Obstacles to pH1N1 Vaccine Availability: The Complex Contracting Relationship among Vaccine Manufacturers, the World Health Organization, Donor and Beneficiary Governments," *The Public Health Response to 2009 H1N1: A Systems Perspective*, eds. Michael A. Stoto, Melissa A. Higdon (New York: Oxford University Press, 2015), p. 207.

951 Ibid.

952 David P. Fidler, Kelley Lee, "Negotiating Equitable Access to Influenza Vaccines: Global Health Diplomacy and the Controversies Surrounding Avian Influenza H5N1 and Pandemic Influenza H1N1."

953 Ibid.

954 "La France veut revendre ses vaccins contre la grippe A." [France wants to sell its vaccines against influenza A] *Le Parisien*, January 3, 2010, <http://www.leparisien.fr/societe/la-france-veut-revendre-ses-vaccins-contre-la-grippe-a-03-01-2010-763246.php>.

955 World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits*, p. 15-16, 18.

economic factors. Lack of demand for influenza vaccines in-country appears to be a particularly important issue facing all producers, which is compounded by a lack of knowledge about optimal vaccination strategies in tropical regions. Therefore, whether current R&D efforts for the establishment of new production lines will come to fruition is uncertain, and the rate of continued development of new production capabilities in the future cannot be ascertained.

US donations of pandemic or seasonal flu vaccines provide a second pathway for GoF-derived benefits to reach developing countries. The US experience during the 2009 H1N1 pandemic demonstrated that although the US was committed to providing some 10% of its vaccine stocks to developing countries through the WHO, the effectiveness of these donations suffered from serious timeliness issues. Although the WHO Pandemic Influenza Preparedness Framework (developed in 2011) established guidelines for vaccine donation during a pandemic in an effort to address these shortcomings, the ability of the US and the WHO to provide donated vaccines in time to mitigate the effects of a high morbidity influenza pandemic in the world's developing countries remains unverified. The US CDC organizes the donation of surplus seasonal influenza vaccines from vaccine manufacturers to developing countries, but several factors significantly limit the impact of this program.

9.14.4 Potential Benefit 2- Assistance in the Development of New Influenza or Coronavirus Antivirals

Several types of GoF research have the potential to inform the development of new influenza or coronavirus antivirals, namely GoF research that alters host tropism, that enhances pathogenicity, and that leads to evasion of antivirals. First, GoF approaches that enhance the virulence of influenza viruses or CoVs may lead to the identification of novel virulence factors that are good therapeutic targets, thereby enabling the development of novel therapeutics. Second, animal-adapted influenza viruses and CoVs developed using GoF approaches that alter host tropism are used for testing the safety and efficacy of candidate therapeutics. Third, GoF approaches that lead to evasion of therapeutics generation information that is recommended for inclusion in an Investigational New Drug (IND) application to the FDA, thereby facilitating regulatory approval of new therapeutics. These benefits may be harnessed by developing countries either through indigenous production of new antivirals, or through direct US donations of antivirals in the event of a pandemic.

9.14.4.1 Capacity for Foreign Production of GoF-Derived New Influenza Antivirals

The process by which a pharmaceutical company abroad can proceed to produce an antiviral compound discovered in the US is complex. When a novel compound showing medical promise is developed into a potential treatment by scientists working for a company, the company typically owns the rights to the discovery as per the scientists' contracts, and is then free to patent the potential treatment.[956] Countries that do not recognize US patents are free to produce the drug provided that no additional bilateral or multilateral trade agreement clauses prohibits this activity. (For example, Tamiflu, which was originally discovered and patented by Gilead Sciences, is not patent protected in Thailand, the Philippines, and Indonesia.)[957,958] For countries where a US patent is legally valid or where a US invention has been patented in-country, domestic producers can either obtain a license or challenge the patent's validity by producing the compound without a license.[959] In practice, firms are often reluctant to license production in order to maintain production line exclusivity, and governmental and public pressure has played a role in

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956 Brian T. Yeh, "Influenza Antiviral Drugs and Patent Law Issues," CRS Report for Congress, August 16, 2007, p. 7, retrieved at http://www.ipmall.info/hosted_resources/crs/RL33159_070816.pdf.

957 Ibid.

958 Roche, "Factsheet Tamiflu," November 17, 2006, p.6, <http://www.roche.com/tamiflu_factsheet.pdf>.

959 Brian T. Yeh, "Influenza Antiviral Drugs and Patent Law Issues,".

convincing US firms to grant licenses to foreign companies. For example, Roche was threatened by several Congress representatives with a temporary abrogation of the Tamiflu patent when the firm was unable to meet demand during the 2005 H5N1 pandemic preparedness period, after which the company reached a number of sub-licensing agreements with other companies abroad to produce the compound.[960] Indeed, national patent law traditionally allows governments to cancel medication patents or to force the licensing of the compounds in response to medical emergencies.[961]

Patents protect a product for a significant period of time. For example, the first US patent covering Tamiflu was filed in 1996 by Gilead Sciences, and the company is still fighting in court attempts to produce generic oseltamivir medication by referencing its patent protections.[962,963] Once associated patents on a compound and its manufacturing expire, all competitors are allowed to produce the compound as a generic medication.[964]

The following section assesses the ability of foreign countries to establish production lines for notional influenza or CoV antivirals developed in the US, based on case studies involving existing influenza antivirals. As highlighted by the discussion above, deriving benefits from such a US discovery relies not only on a foreign country's capacity to establish a production line but also its ability to negotiate complex patent issues. Of note, the conclusions herein are based in the current state of patent and licensing laws. These laws may change as the result of growing public and governmental pressure for affordable medication at the national level, which has stimulated comprehensive multinational trading negotiations that would potentially make it easier for pharmaceutical companies to obtain patents.[965]

9.14.4.1.1 Capacity for Novel Influenza Antiviral Production Abroad

To evaluate the capacity of developing countries to establish production lines for new antivirals, the globalization of production capabilities for the existing influenza antivirals zanamivir, oseltamivir, and peramivir (approved for use in the US), as well as for laninamivir octanoate (approved for use in Japan) are used as case studies to estimate the length of time needed to establish production of a new antiviral. Of note, all four antivirals are small molecule compounds, and all were discovered in high-income (developed) countries. The development timelines for each antiviral compound are summarized in Table 9.4.

960 Brian T. Yeh, "Influenza Antiviral Drugs and Patent Law Issues," p. 3-4.
961 Donald G. McNeil Jr., "Indian Company to Make Generic Version of Flu Drug Tamiflu," *The New York Times*, October 14, 2005, <http://www.nytimes.com/2005/10/14/health/indian-company-to-make-generic-version-of-flu-drug-tamiflu.html>.
962 Kali Hays, "Gilead Sues Lupin Over Plans To Produce Generic Tamiflu," *Law 360*, September 17, 2015, <http://www.law360.com/articles/703920/gilead-sues-lupin-over-plans-to-produce-generic-tamiflu>.
963 U.S. Patent 5,763,483 A, "Carbocyclic Compounds," Filed December 27, 1996, Published June 9, 1998, <http://www.google.com/patents/US5763483>.
964 World Health Organization (WHO), "Generic Drugs," <http://www.who.int/trade/glossary/story034/en/>.
965 "Hard pills to swallow," *The Economist*, January 4, 2014, <http://www.economist.com/news/international/21592655-drug-firms-have-new-medicines-and-patients-are-desperate-them-arguments-over>.

Table 9.4. Information on Influenza Antivirals

Generic name	Proprietary manufacturer[966]	Brand name	Category	Year compound published	Earliest FDA approval, any formulation
Zanamivir	GlaxoSmithKline	Relenza	Neuraminidase inhibitors	1993.[967]	July 1999.[968]
Oseltamivir	Roche	Tamiflu	Neuraminidase inhibitors	1997.[969]	October 1999.[970]
Peramivir	Biocryst	Rapivab	Neuraminidase inhibitors	2000.[971]	Emergency use in 2009, approved for use in December 2014.[972]
Laninamivir octanoate	Biota Pharmaceuticals and Daiichi Sankyo	Inavir	Neuraminidase inhibitors	2009.[973]	Currently not FDA-approved; approved for use in Japan against Influenza A and B since 2010 and 2013, respectively.[974]

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966 [WHO] Technical Studies Under Resolution WHA63.1, Final Document, A/PIP/OEWG/3/2, p. 117;
"Biota Reports That Laninamivir Octanoate is Approved for the Prevention of Influenza in Japan," *Biota*, December 20, 2013, http://investors.biotapharma.com/releasedetail.cfm?releaseid=815483.

967 Mark Von Itzstein et al., "Rational Design of potent sialidase-based inhibitors of influenza virus replication," *Nature* 363 (June 1993): p. 418-423, http://www.nature.com/nature/journal/v363/n6428/abs/363418a0.html.

968 U.S. Food and Drug Administration, "FDA Approved Drug Products: Drug Details, RELENZA," http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails.

969 Kim C. U. et al., "Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity," *J. Am. Chem Soc.* (January 1997): p. 681-690, <http://www.ncbi.nlm.nih.gov/pubmed/16526129>.

970 U.S. Food and Drug Administration, "FDA Approved Drug Products: Drug Details, TAMIFLU," http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails.

971 Babu Y.S. et al., "BCX-1812 (RWJ-270201): discovery of a novel, highly potent, orally active, and selective influenza neuraminidase inhibitor through structure-based drug design," *Journal of Medical Chemistry* 43, no. 19 (2000): p. 3482-3486.

972 U.S. Food and Drug Administration, "FDA approves Rapivab to treat flu infection," *FDA News Release*, December 22, 2014, http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427755.htm.

973 Makoto Yamashita et al., "CS-8958, a Prodrug of the New Neuraminidase Inhibitor R-125489, Shows Long-Acting Anti-Influenza Virus Activity," *Antimicrobial Agents and Chemotherapy* 53, no. 1 (2009): p. 186-192.

974 Biota Pharmaceuticals, Inc., "Biota Provides Update on BARDA Contract for Laninamivir Octanoate," May 8, 2014, http://investors.biotapharma.com/releasedetail.cfm?releaseid=846423.

All four compounds have been produced by some middle-income developing countries. Since companies mostly do not report on R&D efforts nor publicize the terms regarding technology transfers of sublicenses, finding out the average length of time necessary to establish production capability for a given degree of technology assistance is very difficult. Efforts to develop production capabilities in developing countries can nevertheless be broadly grouped into three strategies: licensed activities coupled with follow-on research, independent ventures, and exploratory research in advancing of licensing or the expiration of patents. Some examples of companies in middle-income countries are given below for each strategy to qualitatively illustrate the challenges and timescale associated with each approach, although limited details are available for some cases.

Licensed activities coupled with follow-on research

In China, the Shanghai Pharmaceutical Group and HEC Pharm Co. are the two companies licensed to supply the Chinese state with oseltamivir.[975,976] Under a restriction imposed by Roche, the producers can "only use it for pandemic purposes within China"; in practice, the firms were not allowed to sell the compound commercially and had to furnish oseltamivir to the state at regulated prices.[977] Shanghai Pharmaceutical Group announced they could produce 200,000 doses in *six months* when they obtained their licensing agreement in December 2005.[978] The amount of R&D time invested by the firm prior to December 2005 to establish this oseltamivir production capacity was not revealed, but the announcement came some eight years after oseltamivir was identified as a potential MCM in the published literature (1997).[979]

Also in China, the firm Nanjing Simcere Dongyuan Pharmaceutical Co. Ltd., a subsidiary of Simcere Pharmaceutical Group, obtained a license to produce and sell zanamivir in September 2006.[980,981] According to a Simcere spokesman, GlaxoSmithKline licensed the production of the drug but only provided "limited technical support" in its synthesis.[982,983,984] Thus, a pathway was developed in-country through joint academic-industry research.[985] The firm obtained approval from the Chinese national regulator to manufacture and sell the compound in China in 2010, and the firm is currently selling the compound.[986]

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975. Kirby Chien, Devidutta Tripathy, "China, India drug firms say primed for swine flu," *Reuters*, April 30, 2009, http://uk.reuters.com/article/2009/04/30/us-flu-drugs-generic-idUKTRE53T0UL20090430.
976. "Roche licenses China firm to produce Tamiflu," *China Daily*, December 12, 2005, p.1-2, http://www.chinadaily.com.cn/english/doc/2005-12/12/content_502758.htm.
977. Roche opens Tamiflu to outside firms," *Swiss Info*, December 12, 2005, http://www.swissinfo.ch/eng/roche-opens-tamiflu-to-outside-firms/4900404.
978. Wang Xu, "Shanghai firm wins license for generic version of Tamiflu," *China Daily*, December 13, 2005, http://www.chinadaily.com.cn/english/cndy/2005-12/13/content_502775.htm.
979. Kim C. U. et al., "Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity," *J. Am. Chem Soc.* (January 1997): p. 681-690, http://www.ncbi.nlm.nih.gov/pubmed/16526129.
980. GlaxoSmithKline, "Agreement to increase availability of Zanamivir supply in Asia and Lease Developed Countries," May 15, 2007, http://www.gsk-china.com/asp/News/client/newconten/515200791555.htm.
981. PR Newswire, "Simcere Receives SFDA Approval to Manufacture and Sell Zanamivir in China," *Bloomberg*, February 11, 2010, http://www.bloomberg.com/apps/news?pid=21070001&sid=aRO5.9_34evg.
982. Ibid.
983. "Scientists develop ways producing anti-bird flu drug Zanamivir," *People's Daily*, February 6, 2009, http://en.people.cn/90001/90781/90878/6587151.html.
984. EffactiPharm, "Research Progress," July 10, 2015, <http://www.effectipharm.com/yifang_e.html>.
985. Shanghai Institute of Materia Medica, Chinese Academy of Sciences, "The New Drug Certificate for Anti-H1N1 Flu Medicine Zanamivir granted to SIMM," March 17, 2010, http://english.simm.cas.cn/rp/201003/t20100317_51500.html.
986. Simcere, "Zanamivir," http://www.simcere.com/english/products/detail.asp?gongs_id=59&leibieid=APIs.

Independent ventures

The Indian company Cipla publicly announced in October 2005, during the heightened H5N1 pandemic preparedness period, that it would independently produce oseltamivir without entering into a commercial agreement with Roche.[987] In a subsequent interview, the company chair declared that the company had begun researching oseltamivir production techniques in 2004.[988] In India today, Cipla Ltd., Ranbaxy Laboratories, Strides Arcolab, and Natco Pharma all have production capacity for oseltamivir without having entered into an agreement with Roche.[989,990,991,992,993]

Thailand took advantage of the fact that Tamiflu had not been patent-protected in-country and has had independent production capacity for the generic oseltamivir since 2006.[994,995,996] The Governmental Pharmaceutical Organization manufactured 200,000 tablets in early February 2006, following an announcement that it would do so in December 2005.[997]

Independent exploratory research

A number of research groups in developing countries publish research on synthesis pathway optimization for newly discovered antiviral compounds. The ultimate objective of this type of research may be to prepare for in-country industrial production of the antiviral in question, although end-use intent cannot be definitely predicted based on publications in the scientific literature.

The chemical compound peramivir (first published in 2000 and approved for emergency use in the US in 2009 and for general use in 2014) has already been synthesized in a novel process by a Chinese research team, which achieved this result by March 2012 at the latest.[998] Unlike earlier publications that described known pathways to obtain peramivir that were funded through grants for basic research projects on new

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987 "The Tamiflu Manufacturing Controversy: An Interview with Yusuf Hamied," *Multinational Monitor* vol. 27, no. 2, March/April 2006, http://www.multinationalmonitor.org/mm2006/032006/interview-hamied.html.

988 Ibid.

989 "Resistant strain of swine flu feared; virus killing thousands in India," *Japan Times*, February 26, 2015, http://www.japantimes.co.jp/news/2015/02/26/asia-pacific/science-health-asia-pacific/resistant-strain-of-swine-flu-feared-virus-killing-thousands-in-india/#.VcjldfnZViY.

990 "Swine flu: Hetero Healthcare increases Fluvir production by 400%," *The Economic Times*, February 26, 2015, http://articles.economictimes.indiatimes.com/2015-02-26/news/59541921_1_swine-fluvir-oseltamivir.

991 Khomba Singh, "Govt curbs sale of flu drug Zanamivir," *The Economic Times*, August 29, 2009, http://articles.economictimes.indiatimes.com/2009-08-29/news/28483297_1_swine-flu-drug-oseltamivir-zanamivir.

992 Kirby Chien, Devidutta Tripathy, "China, India drug firms say primed for swine flu," *Reuters*, April 30, 2009, http://uk.reuters.com/article/2009/04/30/us-flu-drugs-generic-idUKTRE53T0UL20090430.

993 "Ranbaxy to supply oseltamivir capsules to US," *The Economic Times*, October 21, 2007, http://articles.economictimes.indiatimes.com/2007-10-21/news/28461984_1_capsules-domestic-sales-generic-version.

994 "Tamiflu- Oseltamivir Production," *News Medical*, February 1, 2011, http://www.news-medical.net/health/Tamiflu-Oseltamivir-Production.aspx.

995 Pennapa Hongthong, "Scientists produce generic Tamiflu," *The Nation*, August 4, 2006, http://www.nationmultimedia.com/2006/08/04/national/national_30010320.php.

996 Roche, "Factsheet Tamiflu," November 17, 2006, p.6, http://www.roche.com/tamiflu_factsheet.pdf.

997 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006, Accessed March 15, 2016.

998 Fei Jia, Jitan Hong, Ping-Hua Sun, Jian-Xin Chen, Wei-Min Chen, "Facile Synthesis of the Neuraminidase Inhibitor Peramivir," *Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry* 43, no. 19 (2013): p. 2641-2647, http://www.tandfonline.com/doi/abs/10.1080/00397911.2012.729279.

drugs,[999,1000] the Chinese research team developed a new pathway designed for *industrial* production. These results demonstrate that domestic production of the compound is well within China's technical capabilities. The peramivir case is one in which a novel synthetic pathway for a US designed chemical was rapidly developed abroad, indeed even before the compound was approved for general use in the US by the FDA. Similarly, in December 2014, a Chinese research team published a novel synthetic pathway for the production of laninamivir octanoate.[1001]

As demonstrated by the above accounts, indigenous production of all four licensed influenza antivirals has been pursued in middle-income countries, to varying degrees and through a variety of mechanisms. Namely, indigenous production lines for zanamivir and oseltamivir have been established in several countries, and Chinese research groups have demonstrated the capability to efficiently synthesize peramivir and laninamivir octanoate, presumably in preparation for the eventual development of production lines in-country. Although the amount of R&D time invested by each of the companies and research teams named above to achieve their production capability is unknown (i.e., when the company began researching synthetic pathways and/or began setting up production facilities), conservative estimates demonstrate that at least some middle-income countries achieved the capacity for full-scale production of a given MCM less than ten years after the compound was initially published in the literature. Notably, several companies rapidly activated large-scale production capabilities in less than six months in 2005–2006 when their governments were preparing for a potential H5N1 pandemic. This suggests that, as with influenza vaccines, a general lack of demand for influenza antivirals appears to be keeping production line globalization in check. Based on these cases, the actual time needed to initiate commercial production of an antiviral designed in a developed country appears to be in the one to five year range.

Of note, barriers to the establishment of antiviral production lines are likely to vary between different types of therapeutics (e.g., small molecule drugs versus monoclonal antibodies), though patenting and licensing issues are likely to be the same for all types.

9.14.4.2 US Antiviral Donations

GoF benefits to the development of novel antivirals may also globalize through US donations of antivirals to developing countries. Current US government assistance to antiviral supply abroad are primarily limited to plans for donations to the WHO for redistribution to developing countries in case of an influenza pandemic. As a member state in the WHO Pandemic Influenza Preparedness Framework, the United States government is committed to contributing influenza antivirals to the WHO-organized Pandemic Influenza Preparedness Benefit Sharing System, which would redistribute MCMs to third countries as part of a pandemic response as needed.[1002] US private pharmaceutical companies can and have donated antiviral treatments to the WHO and to countries dealing with local outbreaks independently

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[999] 顾轶娜,林东海,"新型抗流感病毒神经氨酸酶抑制剂帕拉米韦研究进展,"中国生化药物杂志 30, no. 4 (2009): p.273-276 [GU Yi-na, LIN Dong-Hai, "Research progress on peramivir as a novel anti-influenza virus neuraminidase inhibitor," *Chinese Journal of Biochemical Pharmaceutics* 30 no. 4 (2009): p.273-276.].

[1000] 贾飞, 陈良柱, 陈建新, 孙平华, 陈卫民,"帕拉米韦合成路线图解,"中国医药工业杂志 42 no. 12 (2011): p. 954-956. [JIA Fei, CHEN Jianxin, SUN Pinghua, CHEN Weimin, "Graphical Synthetic Routes of Peramivir," *Chinese Journal of Pharmaceuticals* 42, no. 12 (2011): p. 954-956.]

[1001] Tian J. et al., "Organocatalytic and scalable synthesis of the anti-influenza drugs zanamivir, laninamivir, and CS-8958," *Angewandte Chemie* 126 (2014): p. 14105-14108.

[1002] World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits*, p. 15-16, 18.

from government contributions.[1003,1004] However, these private companies are under no obligation to do so in the future, and hence the effect of this potential GOF-derived benefits dissemination pathway cannot be reliably assessed.

As there are no licensed therapeutics for coronaviruses in the US or abroad, neither the US nor the WHO have formal policies or plans in place for the donation of (notional) therapeutics in the event of an epidemic caused by a novel coronavirus.

The following case study reviews US donations of antivirals to foreign countries during the 2009 H1N1 pandemic and identifies bottlenecks that may pose a barrier to the globalization of GoF benefits via this pathway in the future. Although the creation of the WHO Pandemic Influenza Preparedness (PIP) Framework in 2011 limits the extent to which this case study is predictive of the successes and challenges of influenza antiviral donation efforts in the future given its plan for a joint pre-pandemic influenza antivirals stockpile,[1005] similar challenges could be encountered in the event of ad hoc donation of CoV therapeutics during a CoV epidemic.

During the 2009 H1N1 pandemic, the US initially donated 400,000 antiviral treatment courses to Mexico, followed by 420,000 courses of oseltamivir for the Pan American Health Organization (PAHO).[1006] PAHO then provided stocks to countries throughout Latin America and the Caribbean.[1007] Although this demonstrates US willingness to provide antiviral doses in the event of a pandemic, one US public health policy stakeholder stated that the global health security enterprise may not be as willing to donate antivirals in the event of future pandemics due to the expense associated with storing and deploying the drugs.[1008] The use of donated antivirals during the H1N1 pandemic in developing countries was in general suboptimal, in part due to the low availability of the antiviral compounds in recipient countries.[1009] In Asia, for instance, an authoritative review article noted that "health practitioners were reluctant to follow the recommendation of the empiric use of oseltamivir"; the practitioners did not wish to use scarce doses on ostensibly mild cases of influenza, even when the patient was in a high-risk group.[1010]

In sum, although US policy supports the donation of influenza antivirals in the event of a pandemic, the relatively small number of doses donated in comparison to the global need in the event of a pandemic means that developing countries would face shortages, which would in turn exacerbate poor usage in-country.

1003 David Reddy, "Responding to pandemic (H1N1) 2009 influenza: the role of oseltamivir," *J. Antimicrob. Chemother.* 65 supplement 2 (April 2010): ii35-ii40, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835510/pdf/dkq014.pdf>.

1004 Roche, "Factsheet Tamiflu," November 17, 2006, p.6, http://www.roche.com/tamiflu_factsheet.pdf.

1005 World Health Organization (WHO), Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits (Geneva: World Health Organization Press, 2011), p. 18, http://apps.who.int/iris/bitstream/10665/44796/1/9789241503082_eng.pdf.

1006 "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," p. 38, http://www.phe.gov/Preparedness/mcm/h1n1-retrospective/Documents/h1n1-retrospective.pdf.

1007 United States of America, "Identifying and addressing barriers to the emergency sharing of international public health and medical assistance," Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, Meeting of Experts, Geneva, Switzerland, August 12-16, 2013, BWC/MSP/2013/MX/WP.6, p. 2 para. 5.

1008 Interview with US government official involved in public health preparedness and response decision-making for influenza outbreaks.

1009 Dale Fisher et al. "Pandemic response lessons from influenza H1N1 2009 in Asia," *Respirology* 16 (2011): p. 879, http://onlinelibrary.wiley.com/doi/10.1111/j.1440-1843.2011.02003.x/abstract.

1010 Ibid.

9.14.4.3 Summary – Globalization Potential of GoF Benefits to Influenza Vaccine Production

GoF research has the potential to benefit the development of novel therapeutics for influenza viruses and coronaviruses. The ability of developing countries to establish production lines for such new antivirals depend not only on their manufacturing capabilities but also on their ability to negotiate the complex patent issues surrounding the marketing of therapeutics. In cases where patent protections do not apply, case studies of international production of licensed influenza antivirals suggest that the time needed to initiate commercial production of a US-designed antiviral is one to five years. Patent protections do not apply when a patent is not recognized nationally or is abrogated during a medical emergency, or where the compound can be sublicensed from the patent owner. Notably, several companies in developing countries rapidly activated influenza antiviral production capabilities to produce hundreds of thousands of doses in less than six months in 2005–2006, when their governments were preparing for a potential H5N1 pandemic. This capacity for rapid scale-up of production suggests that the actual time needed for establishment of a new production line may be much less than five years. As with influenza vaccines, a general lack of domestic demand for influenza antivirals appears to be keeping globalization of GoF benefits related to the development of novel therapeutics in check.

The US demonstrated its willingness to donate antivirals during the 2009 H1N1 pandemic. However, problems of timeliness of supply compounded issues of suboptimal use in-country. The WHO Pandemic Influenza Preparedness Framework (developed in 2011) addresses these shortcomings but remains untested.

9.14.5 Potential Benefit 3- Benefits to Pandemic Preparedness Planning

This section assesses the globalization of GoF benefits that inform pandemic preparedness planning, which includes two benefits. First, the demonstration that avian influenza viruses can evolve the capacity for more efficient transmission in mammals may, in and of itself, stimulate interest and investment in pandemic preparedness initiatives. Second, molecular markers for phenotypic properties of concern (e.g., virulence, transmissibility, mammalian adaptation, and antiviral resistance), which are discovered and validated using GoF approaches, inform pandemic risk assessments that guide prioritization of resources for pandemic preparedness activities. The first benefit derives from GoF research that enhances the transmissibility of influenza viruses in mammals, and the second derives from GoF research that enhances the infectivity or transmissibility of influenza viruses in mammals, that enhances the virulence of influenza viruses, and that leads to evasion of influenza viruses from therapeutics.

The extent to which these GoF benefits will globalize depends on whether and how information derived from GoF studies influences decision-making about pandemic preparedness activities in countries in which high-risk animal influenza viruses are circulating, as well as whether these countries have the ability to engage in pandemic preparedness initiatives.

9.14.5.1 Role of GoF Research in Pandemic Risk Assessments for Developing Countries

First, the role of GoF research in pandemic risk assessments conducted by developing countries is assessed. Two types of GoF studies are considered: (1) ""proof of principle" demonstrations that particular animal influenza viruses can acquire pandemic properties (e.g., transmissibility) in the laboratory and (2) studies that establish molecular markers for phenotypic properties of concern (transmissibility, virulence, etc.).

Although "proof of principle" experiments that demonstrate that an avian virus (e.g., H5N1) can acquire the capacity for more efficient transmission in mammals have had minimal impacts on USG initiatives due to the already high investments in pandemic preparedness, these GoF results have relatively greater impacts on preparedness efforts in developing countries. One international public health official stated that the experimental demonstration that H5N1 could evolve the capacity for airborne transmission in ferrets was of "great importance" in countries where H5N1 was circulating.[1011,1012,1013] In response, some countries mounted communications campaigns to engage with the public, public health personnel, and health care workers about the risks associated with H5N1, in an effort to bolster their surveillance capabilities. Thus, to date, these GoF experiments primarily benefit global rather than domestic populations.

Most developing countries in which animal influenza viruses of concern (e.g., H5N1) are circulating are not capable of conducting ferret experiments to evaluate the transmissibility and virulence of viruses, which contribute critical data to a pandemic risk assessment (see Section 9.6.3.3).[1014] As a result, those countries carry out risk assessments in conjunction with the WHO (as well as the CDC and other laboratories in the GISRS as needed).[1015] This collaborative relationship is codified in the WHO's Pandemic Influenza Preparedness Benefit Sharing System, which states that WHO will seek to ensure that member states and the WHO Secretariat "provide pandemic surveillance and risk assessment and early warning information and services to all countries."[1016] These assessments are conducted with input from the Ministries of Health in a country of interest.[1017] Similar to risk assessments conducted by the USG, WHO risk assessments consider the presence of molecular markers of mammalian adaptation, transmissibility, and virulence, alongside virological data and in the context of environmental factors that play important roles in the emergence of pandemic viruses.

Ultimately, the ability of a developing country to derive benefits from risk assessments informed by GoF research will depend on the ability of the country to engage in responsive pandemic preparedness activities. These include enhanced surveillance, implementation of community-level risk mitigation measures, and pre-pandemic vaccine development.[1018] The following sections assess the potential for developing countries to put in place such "downstream" responses.

9.14.5.2 Capacity for Responsive Public Health Activities in Developing Countries

Responsive capabilities are primarily relevant in countries in which zoonotic influenza viruses (or influenza viruses with zoonotic potential) are currently circulating. As seen on the map below (Figure 9.7), most countries in the world have detected cases of zoonotic avian influenza in humans or in birds

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1011 Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

1012 Imai M *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486: 420-428

1013 (2015g) Interview with international researcher or international public health official.

1014 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

1015 Ibid.

1016 World Health Organization (WHO), *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits*, p.15.

1017 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

1018 C. Todd Davis et al., "Use of Highly Pathogenic Avian Influenza A(H5N1) Gain-Of-Function Studies for Molecular-Based Surveillance and Pandemic Preparedness," *mBio* 5, no. 6 (December 12, 2014) http://mbio.asm.org/content/5/6/e02431-14.full.

within the last five years. Notably, a lack of detected cases may be due to poor detection and reporting capabilities rather than the absence of avian influenza.[1019]

Figure 9.7. Countries that reported a detected case of zoonotic influenza in humans or birds within the last five years.[1020,1021,1022,1023]

Many countries with AI detections are developing (low- or middle-income) countries, in particular most countries with repeated detections (i.e., multiple years) and sustained outbreaks in domestic poultry populations. Public health responses to zoonotic influenza outbreaks in developing countries are particularly challenging due to limited resources for carrying out response activities and because of the need for a strong and coordinated veterinary service – public health system. The veterinary services of most developing countries greatly suffer from weak human organizational factors compounded by resource constraints.[1024] The lack of effective communication strategies for behavioral interventions that will reduce risks of disease spillover (e.g., at poultry farms, live bird markets, etc.) was also highlighted by influenza researchers and public health experts as a major challenge.[1025] Convincing the public to

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1019 Tiaji Salaam-Blyther, "The 2009 Influenza Pandemic: U.S. Responses to Global Human Cases," Congressional Research Service, June 23, 2009, p. 11, https://www.acs.org/content/dam/acsorg/policy/acsonthehill/globalchallengesdiscussions/swineflu/crs-r40588-us-responses.pdf.

1020 H5N1, H5N6, H6N1, H7N2, H7N3, H7N7, H7N9, H9N2, H10N7, H10N8.

1021 World Health Organization (WHO), "Disease Outbreak News (DONs)," <http://www.who.int/csr/don/en/>.

1022 World Health Organization (WHO), "Monthly Risk Assessment Summary, Influenza at the Human-Animal Interface," http://www.who.int/influenza/human_animal_interface/HAI_Risk_Assessment/en/.

1023 Food and Agriculture Organization of the United States, "EMPRES-i Global Animal Disease Information System," http://empres-i.fao.org/eipws3g/.

1024 J. Weaver et al., "Initial assessment of strategic plans for improving the performance of Veterinary Services in developing countries: a review of OIE PVS Gap Analysis reports," *Rev. sci. tech. Off. int. Epiz.* 32, no. 2 (2012): p. 631-645.

1025 (2015g) Interview with international researcher or international public health official.

comply with disruptive measures is difficult, and one expert noted the value of GoF research results in strengthening the evidence basis for recommendations.

These challenges are highlighted by Vietnam's response to a series of H5N1 outbreaks in poultry in 2004 – 2005, which led to multiple cases of human infection. Vietnam's initially responded by eradicating infected birds and implementing movement restrictions for poultry, which proved to be ineffective given their lack of nationwide surveillance and coordinated response capabilities.[1026] Vietnam then launched a nationwide surveillance effort and institution a mass vaccination program for poultry. These measures also met with limited success, due to problems with recognition and reporting systems, insufficient collaboration between human and animal health sectors, a general lack of resources to implement "active surveillance and research" and other factors.[1027,1028] Today, H5N1 is considered endemic in poultry in Vietnam, and sporadic cases of human infection with H5N1 continue to be reported by Vietnam.[1029]

In contrast, Thailand, which also experienced H5N1 outbreaks in poultry and human infections during that same time period, was able to mount a robust public health response that eradicated the virus from domestic poultry production systems.[1030] The Thai government implemented enhanced surveillance for human and poultry cases, coupled with aggressive measures to eradicate the virus from poultry operations, including culling of infected birds, destruction of related productions (e.g., feed, bedding, etc.), and poultry movement controls.[1031,1032] In addition, the government produced and sold oseltamivir tablets at subsidized prices, starting with 200,000 tablets manufactured in February 2006.[1033] As a result of these response measures, the last reported human case of avian influenza in Thailand was in 2006 and the last reported animal case of avian influenza was in 2008.[1034,1035,1036]

These case studies demonstrate the overarching importance of a strong public health sector in being able to benefit from pandemic risk assessments through implementation of prevention activities. Importantly,

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1026 Ricardo J. Soares Magalhaes, Dirk U. Pfeiffer, Joachim Otte, "Evaluating the control of HPAIV H5N1 in Vietnam: virus transmission within infected flocks reported before and after vaccination," *BMC Vet Res.* 6 (2010): p.1 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2898779/pdf/1746-6148-6-31.pdf.

1027 Xiu-Feng Wan et al., "Evolution of Highly Pathogenic H5N1 Avian Influenza Viruses in Vietnam between 2001 and 2007," *PloSOne* 3, no. 10 (October 2008): 1-12, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2565130/pdf/pone.0003462.pdf.

1028 Nguyen Tran Hien, "Avian Influenza In Vietnam: Situation and Lessons Learned," p.17, http://www.fao.org/docs/eims/upload/250718/aj167e00.pdf.

1029 Sharmi W. Thor et al., "Detection and Characterization of Clade 1 Reassortant H5N1 Viruses Isolated from Human Cases in Vietnam during 2013," *PloS One* 10, no. 8 (2015): p. 1-20, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4526568/pdf/pone.0133867.pdf.

1030 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1031 Thanawat Tiensin et al., "Highly Pathogenic Avian Influenza H5N1, Thailand, 2004," *Emerging Infectious Diseases* 11, no. 11 (November 2005), http://wwwnc.cdc.gov/eid/article/11/11/05-0608_article.

1032 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1033 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1034 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1035 OIE, World Animal Health Organization Database (WAHID), "Detailed Country(ies) disease incidence," http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail.

1036 Food and Agriculture Organization of the United States, "EMPRES-i Global Animal Disease Information System," http://empres-i.fao.org/eipws3g/.

the example of Thailand highlights that a robust response to a significant public health risk in middle-income countries is not impossible.

9.14.5.3 Capacity for Pre-Pandemic Vaccine Production

In addition to implementing community-level prevention and surveillance activities in response to a high-risk pandemic risk assessment, developing countries could derive benefits from such assessments by investing in pre-pandemic vaccine development and stockpiling. The influenza vaccine producers with influenza vaccines on the market identified in developing countries (see Section 16.9.6) are all capable of producing pandemic vaccine strains using CVVs obtained through the WHO framework, as explained in Section 9.14.3.1 above. The map in Figure 9.8 shows an overlay of the developing countries with current vaccine production capabilities and those in which zoonotic influenza viruses have been detected in bird and/or human populations within the past five years. Only seven out of 28 developing countries with zoonotic AI detections in humans or in bird populations over the past five years have the capacity to produce vaccines in-country. This result highlights that a limited number of countries that may be at risk of the emergence of a novel pandemic strain within their borders can benefit from pandemic risk assessments through the development and stockpiling of pre-pandemic vaccines. Notably, WHO does not stockpile pre-pandemic vaccines for use in developing countries, but is rather focused on ensuring real-time access to pandemic vaccines during a pandemic as outlined in the Pandemic Influenza Preparedness Framework.[1037,1038]

[1037] Immunizations SWGoIVa. Influenza A (H5N1) Vaccine Stockpile and Inter-Pandemic Vaccine Use Background Document. http://www.who.int/immunization/sage/meetings/2013/november/SAGE_WG_H5vaccine_background_paper_16Oct2013_v4.pdf. Last Update Accessed October 31, 2015.

[1038] World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits* (Geneva: World Health Organization Press, 2011), p. 15-16, 18.

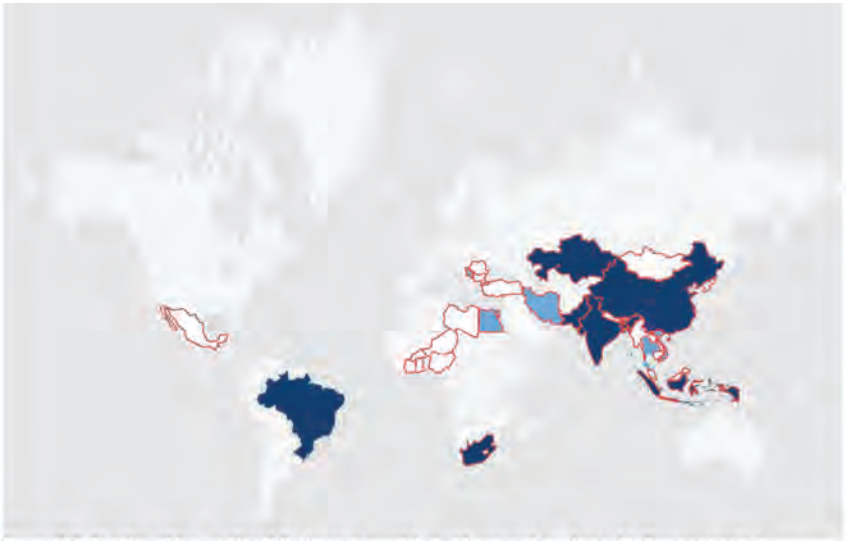

Figure 9.8. Overlay of low- and middle-income countries with current or planned influenza vaccine production capabilities and those that have reported AI detections in birds to OIE within the past five years. Regions with AI detections are outlined in red. Countries (or regions) without vaccine production capabilities are shaded in white, countries with current vaccine production capabilities are shaded in dark blue, and countries with planned vaccine production lines are shaded in cyan.

9.14.5.4 Summary – Globalization of GoF Benefits That Inform Pandemic Risk Assessments

The demonstration that animal influenza viruses can acquire pandemic properties in a laboratory setting may galvanize preparedness efforts in developing countries where the virus is circulating in agricultural animal or wildlife populations. For example, the 2012 demonstration that H5N1 could evolve the capacity for airborne transmission between ferrets triggered some developing countries to initiate communications campaigns to raise awareness of the risks associated with H5N1 infections among the public, public health personnel, and healthcare workers, in order to bolster early detection capabilities.

Because most developing countries in which high-risk animal influenza viruses are circulating lack the capabilities to conduct ferret experiments evaluating the transmissibility and virulence of viruses, data which critically inform pandemic risk assessments, risk assessments are carried out in collaboration with the WHO and laboratory members of the GISRS (including the CDC). Similar to USG risk assessments, these risk assessments incorporate information derived from GoF research, alongside epidemiologic and virologic data, and environmental factors that influence the pandemic potential of the virus.

Downstream of a pandemic risk assessment, the ability of developing countries to implement prevention and early detection measures in response to the detection of zoonotic influenza cases or outbreaks in humans and/or animals varies widely, depending on the state of public health infrastructure, the relationship between the Veterinary Services and Public Health sectors, and the resources for investing the prevention and response activities. Thailand's ability to eradicate H5N1 from their poultry production

system in response to widespread outbreaks in poultry populations as well as multiple human spillover cases in 2003 – 2006 indicates that successful eradication campaigns are possible. However, the fact that Vietnam continues to experience HPAI outbreaks since the initial 2004 – 2005 outbreak in the region highlights the challenges for successfully carrying out response activities that mitigate the risk of avian influenza spillover into human populations.

Although multiple developing countries in which zoonotic avian influenza infections have been detected in human and/or bird populations within the past five years currently have the capacity to produce pre-pandemic influenza vaccines in-country, 21 do not. As WHO does not stockpile pre-pandemic vaccines, the lack of vaccine production capabilities in some at-risk countries limits the globalization potential of GoF benefits related to pandemic risk assessments.

10 Potential Proliferation of GoF Research

10.1 Summary

The risks associated with GoF research are proportional to the size of the research community engaged in this research. Consequently, we must estimate how many laboratories may be performing GoF experiments if the moratorium is lifted, given the availability of personnel, facilities, and resources. Using publication and funding data, we identified a group of 40 active, well-funded researchers in the US who have been performing, or have the capacity to perform, the experiments that meet the definition of GoF research. Hundreds of BSL-3 containment facilities in the US and the level of NIH funding for influenza, SARS, and MERS research offers potential for growth. Using historical examples, we showed that a new discovery in this field may proliferate to as few as one and as many as 70 new groups around the world within 10-15 years, of which approximately half have no authorship connection to the founding groups.

10.2 Purpose and Approach

The goal of this task was to estimate the expansion potential of Gain of Function research if the United States Government funding pause is lifted. Simply put, we are trying to answer the question of how many labs may be participating in GoF research in the next few years given the state of the field today. This information is important for risk estimates, because the probability of most laboratory incidents is proportional to the number of groups performing these experiments. Research expansion, which we also call proliferation, depends on three factors:

1. Size of the interested and capable research community,
2. Availability of resources to conduct the research, and
3. The rate and extent of discovery uptake by the research community.

We aimed to quantify each of these factors. Interest in the research community was measured by the number of laboratories that published GoF studies; availability of resources was based on the NIH funding levels and number of BSL-3/4 facilities; and rate and extent of proliferation was estimated using historical examples of discoveries in virology approximating GoF research.

10.3 Methods

10.3.1 Definition

This analysis was based on the types of the GoF research recommended for assessment by the National Science Advisory Board for Biosecurity (NSABB),[1039] as follows:

- Pathogens included – seasonal influenza, highly pathogenic avian influenza virus H5N1, low pathogenic avian influenza virus H7N9, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and
- Pathogen characteristics – enhanced pathogen production as a result of changes in the replication cycle or growth, enhanced morbidity and mortality in appropriate animal models, enhanced transmission in mammals, evasion of existing natural or induced immunity, resistance to drugs or evasion of other medical countermeasures such as vaccines, therapeutics, diagnostics.

[1039] Framework for Conducting Risk and Benefit Assessment of Gain-of-Function Research: Recommendations of the National Advisory Board for Biosecurity. May 2015. http://osp.od.nih.gov/sites/default/files/resources/NSABB_Framework_for_Risk_and_Benefit_Assessments_of_GOF_Research-APPROVED.pdf

10.3.2 Key Informant Interviews

Interviews were conducted with eleven GoF researchers, with whom we discussed the following questions:

1. How many groups represent the GoF research community in the US?
2. How many BSL-3 and BSL-4 facilities are currently available to conduct GoF research in the US?
3. In what way has the moratorium and the dialogue surrounding GoF research influenced your group's interest in doing this work in the future?
4. Can you suggest a past discovery that would make a meaningful case study for GoF research?

Not all researchers had the same expertise and so did not answer all the questions.

10.3.3 Publication Analysis to Determine Interest

To identify the size of the research community interested in GoF research, we used three methods. First, we searched PubMed and Web of Science (WoS) databases to obtain citations to two studies discussed in the scientific and policy literature as exemplary of GoF research.[1040,1041,1042] Second, we abstracted all publications ranked as similar to these two articles by PubMed.[1043] Finally, we queried PubMed and WoS with search terms that were derived from the NSABB Framework of GoF research, such as "enhanced pathogenicity and influenza virus" and "enhanced transmissibility and SARS virus" (full list of search terms is included in the Appendix I Section 12).[1044] Articles in foreign languages, reviews, book chapters, editorials, opinion pieces, and conference abstracts were excluded. The searches were conducted in June – July 2015 and were limited to the past five years.

For each data set, we abstracted the names of last authors with three or more publications in order to identify the most active groups. The resulting list was de-duplicated and compared to the names of investigators who received notifications under the USG funding pause and the missing names were added to make the final list of PIs.[1045]

10.3.4 Identification and Analysis of Historical Case Studies of Research Proliferation

Our objective was to find three historical examples of discoveries that were made 10-15 years ago that involved a virus, (ideally pandemic influenza, SARS, or MERS) and then analyze to what extent such studies resulted in an expansion of this work. The choice of case studies was informed by key informant interviews. Once the paper describing the initial discovery was identified, we used the approach described

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[1040] Web of Science. https://isiknowledge.com/

[1041] Imai M, *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486(7403):420-8.

[1042] Herfst S, *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science*. 336(6088):1534-41.

[1043] The set of similar articles is generated by comparing words from the title, abstract, and MeSH terms using a word-weighted algorithm. PubMed. http://www.ncbi.nlm.nih.gov/pubmed.

[1044] Framework for Conducting Risk and Benefit Assessment of Gain-of-Function Research: Recommendations of the National Advisory Board for Biosecurity. May 2015.

[1045] Jocelyn Kaiser. 17 November 2014. Moratorium on risky virology studies leaves work at 14 institutions in limbo. http://news.sciencemag.org/biology/2014/11/moratorium-risky-virology-studies-leaves-work-14-institutions-limbo

in the publication analysis section to construct data sets. Each publication on the final list was examined to determine whether it included the following experimental approaches:

- SARS case study – use of animals infected with live virus,
- PB2 case study – use of animals infected with PB2 mutant influenza strains, and
- 1918 case study – use of animals infected with reconstructed 1918 influenza strain.

The articles that did not meet these criteria were excluded. The remaining papers were further examined to exclude the studies that were performed in facilities with containment levels lower than BSL-2+ as these were unlikely to be relevant to proliferation of GoF research because according to the CDC guidelines, propagation of SARS, MERS, and pandemic influenza viruses in cell culture and their use in the inoculation of animals requires BSL-3 containment facilities.[1046,1047,1048] BSL-2+ was included based on the assumption that certain types of experiments that satisfy our inclusion criteria (e.g., with seasonal influenza) can be performed under this containment level. The resulting papers were analyzed for publication year, author names, and author affiliations.

10.3.5 Publication Analysis to Determine Proliferation Path

Dendrograms were constructed based on the relationship between common authors on prior and subsequent papers. In one set of diagrams we mapped the network of last authors who became middle authors and in another the network of middle authors who became last authors. We then estimated percent of authors with and without publication connections.

10.3.6 Analysis of Funding Data

We queried the federal funding database RePORTER using the terms "influenza," "SARS," and "MERS" to obtain total funding levels and with the names of 40 PIs with interest in GoF research to obtain individual-level data. Searches were limited to NIH as a funder[1049] and each hit was examined to ensure its relevance.

10.4 Limitations

Our study had several limitations. First, last authors were used to define a research group, which may not always be accurate. Second, funding data included only the NIH expenditures for research on influenza, SARS, and MERS viruses, resulting in under-estimate. Third, we could not determine how much of the funding is spent on GoF research because publically available grant abstracts do not contain sufficient information. Fourth, it is not possible to establish causality between a publication of the seminal paper and subsequent research efforts by other groups. Fifth, the dendrograms represent only the links between the authors on papers included in each case study. Therefore, they are a snapshot in time and are unlikely to show the full picture of proliferation. Finally, depending on the nature of the discovery and many other factors in the research environment, proliferation may take different paths than what emerged from the case studies.

[1046] http://www.cdc.gov/sars/guidance/F-lab/app5.html
[1047] http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html
[1048] http://www.cdc.gov/flu/avianflu/h7n9/risk-assessment.htm
[1049] NIH Reporter database. http://projectreporter.nih.gov/reporter.cfm

10.5 Findings

10.5.1 Interest in the Research Community

To estimate the level of interest in conducting GoF research, we performed bibliometric analysis. Papers citing either of the two articles that generated influenza viruses transmissible by air in ferrets (Imai et al. and Herfst et al.), papers rated as similar to these articles by PubMed, and hits to the queries presented in Table 10.1 were used as sources of interested groups. These searches resulted in nearly 3000 papers for influenza and 2000 for SARS and MERS (Table 10.1). Removing duplicate publications reduced the number of entries to 1805 and 1558, respectively.

In order to identify active GoF groups, we abstracted the names of all authors with three or more publications over a five-year period. The resulting sets contained 259 influenza and 102 SARS/MERS authors, of which 35 were the last authors and assumed to be Principal Investigators/group leaders. Authors based outside of the US were excluded. The list of 35 authors was compared against the names of the researchers who received notifications from NIH under the moratorium, resulting in the addition of five individuals, bringing the total number to 40 investigators (Table 10.2).[1050] This list represents the group that has research interests and skill sets that align well with GoF research.

Table 10.1. Search Results to Determine Level of Interest in GoF Research

Results	Influenza	SARS/MERS
Total publications	2738	1886
Total unique publications	1805	1558
Unique authors with 3+ pubs	259	102

Table 10.2. Names of Principal Investigators in Alphabetical Order

Baric, Ralph S	Harrod, Kevin S	Mehle, Andrew	Subbarao, Kanta
Bouvier, Nicole M	Harty, John T	Morrey, John D*	Taubenberger, Jeffery K
Compans, Richard W	Heise, Mark T	Orenstein, Walter A*	Tompkins, Stephen M
Denison, Mark R	Katze, Michael G	Palese, Peter*	Topham, David J
Enjuanes, Luis	Kawaoka, Yoshihiro	Pekosz, Andrew*	Treanor, John J*
Feldmann, Heinrich	Lenschow, Deborah J	Perez, Daniel R	Tripp, Ralph A
Frieman, Matthew B	Lowen, Anice C	Perlman, Stanley	Tseng, Chien-Te K
Gallagher, Thomas M	Manicassamy, Balaji	Richt, Juergen A	Tumpey, Terrence M
Garcia-Sastre, Adolfo	Martinez-Sobrido, Luis	Schultz-Cherry, Stacey	Webby, Richard J
Govorkova, Elena A	Mccray, Paul B	Steel, John	Webster, Robert G

**Added based on the moratorium notifications*
Authors based outside of the US were excluded

[1050] Jocelyn Kaiser. Moratorium on risky virology studies leaves work at 14 institutions in limbo. Science Magazine. http://news.sciencemag.org/biology/2014/11/moratorium-risky-virology-studies-leaves-work-14-institutions-limbo.

We also investigated the size of the GoF community via key informant interviews. Of the 11 researchers interviewed, five provided estimates, which ranged from three to 20. One respondent said that it included essentially all influenza researchers. These anecdotal data suggest that our estimate of 40 groups probably represents the upper bound of interested groups.

10.5.2 Availability of Resources

While the interest and skills in the research community are required to conduct GoF research, they are not sufficient without funding support and appropriate containment facilities in which to conduct the experiments. We used RePORTER database to obtain data on NIH funding levels for influenza, SARS, and MERS research. We found that between 2010 and 2014 the NIH expenditures ranged from approximately $56M to $69M for SARS, from $45M to $46M for MERS, and from $596M to $747M for influenza (Table 10.3).[1051,1052] Funding for SARS and influenza decreased and for MERS increased over the period examined, which is consistent with the emerging status of MERS.

Table 10.3. NIH Funding for SARS, MERS, and Influenza Research

FY	SARS	MERS	Influenza
	Dollar amount, million	Dollar amount, million	Dollar amount, million
2010	56	0	747
2011	41	0	597
2012	39	0	596
2013	69	46	677
2014	39	45	654

We also determined the level of NIH funding for the 40 PIs who represent the GoF community (names shown in Table 10.2). The data were collected over a five-year period to minimize year-to-year variation. Figure 10.1 shows that all but four investigators had NIH funding, with the amounts ranging from $250K to over $10M per year. The median funding level for 36 PIs with funding was $1.5M per year. We are unsure why the data show a bimodal distribution with nearly all laboratories supported by grants that total drastically more or less than this median value. According to the NIH estimates, this amount exceeds the total for three average R01 grants.[1053] As a very rough estimate, each R01 can support three researchers and supplies for their experiments. Consequently, based solely on the raw numbers sufficient funding is available to support over 100 additional researchers or approximately 150 researchers in total. However, it is not possible to accurately estimate how much of the available funding currently supports or can support GoF research versus non-GoF research involving SARS, MERS, and influenza viruses.

[1051] We found significant discrepancies in the funding data contained in RePORTER and FederalRePORTER. Because RePORTER is a more established system, we used it as data source. RePORTER contains primarily NIH funding data.

[1052] We found significant inconsistencies in the data in the Reporter and FederalReporter databases in funding amounts and the number of projects. Reporter was ultimately used because it is a more established database.

[1053] https://nexus.od.nih.gov/all/2014/01/10/fy2013-by-the-numbers/

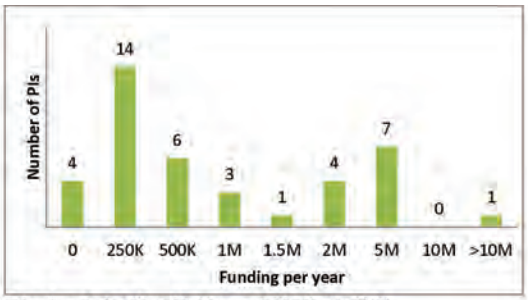

Figure 10.1: NIH Funding Levels for PIs with Research Similar to GoF.

According to the CDC guidelines, propagation of SARS, MERS, and pandemic influenza viruses in cell culture and their use in the inoculation of animals requires BSL-3 containment facilities.[1054,1055,1056] Consequently, availability of these facilities limits the level of research activity that may contribute to biosafety risk. To estimate the upper bound of this limit, we reviewed the literature to determine the number of high containment facilities in the United States. Information on the BSL-4 facilities was available from several sources, and the estimates ranged from five to eight (Table 10.4). In contrast, a completely reliable number of BSL-3 facilities could not found. One source put this number at 1,495 in 2010.[1057] However, according to the GAO report, "no single federal agency has the mission to track and determine the risk associated with the expansion of BSL-3 and BSL-4 labs in the United States, and no single federal agency knows how many such labs there are in the United States."[1058] Importantly, all estimates that we were able to find put the number of laboratories in the hundreds at a minimum, which represents vastly more containment capacity than needed to support the 40 interested groups identified above.[1059,1060]

[1054] http://www.cdc.gov/sars/guidance/F-lab/app5.html
[1055] http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html
[1056] http://www.cdc.gov/flu/avianflu/h7n9/risk-assessment.htm
[1057] Jocelyn Kaiser (2011). Taking Stock of the Biodefense Boom. *Science* Vol. 333 (6047): 1214.
[1058] High-Containment Biosafety Laboratories: Preliminary Observations on the Oversight of the Proliferation of BSL-3 and BSL-4 Laboratories in the United States. GAO-08-108T. 2007.
[1059] USA Today. http://www.usatoday.com/story/news/2015/05/28/biolabs-pathogens-location-incidents/26587505/
[1060] High Containment Laboratories. National Strategy for Oversight is Needed. GAO-09-574. 2009

Table 10.4. BSL-4 Facilities in the United States			
	O=operational NO=not operational E=expanding		
	GAO 2009	Kuhn, presentation	FAS, website
NIAID Rocky Mountain Lab, Hamilton MT	O	O	O
CDC, Atlanta GA	O	O	O
Georgia State University, Atlanta GA	O	O	O
DOD USAMRIID, Fort Detrick MD	O	O	E
University of Texas Medical Branch, Galveston TX	O	O	O
Southwest Foundation for Biomedical, San Antonio TX	O	O	O
DHS National Bio and Agro-Defense Facility, Manhattan KS	NO	N/A	NO
Boston University NBL, Boston MA	NO	N/A	NO
DHS National Biodefense Analysis and Countermeasures Center, Fort Detrick MD	NO	N/A	NO
NIAID Integrated Research Facility, Fort Detrick MD	NO	N/A	NO
Virginia Division of Consolidated Laboratory Services, Richmond VA	NO	O	NO

10.5.3 Other Factors to Influence Proliferation

Based on a small survey, Julie Pfeiffer suggested that the current debate about GoF research and the funding pause are having a negative effect on the career choices of scientists in training.[1061] We explored this phenomenon in key informant interviews with GoF researchers. The majority of respondents (seven out of eleven or 63%) confirmed that the pause has had a "chilling effect" on their trainees. While the interviews were conducted at the affected laboratories and probably represent a negatively biased opinion, the data indicate that future rates of proliferation might be inhibited by workforce shortages due to uncertainty in the ability to conduct the research, negative publicity, or other factors.

10.5.4 Case Studies

To assess how quickly a research discovery, once made, will propagate through the scientific community and lead to additional labs conducting similar research, we used historical discoveries as case studies. Please recall that we sought discoveries that were made in 2000–2005, involving SARS, MERS, or influenza virus, and reasonably expected to lead to GoF research. The following discoveries were proposed by GoF researchers: SARS animal model; growing embryonic stem cells in culture; macaque animal model using chimeric HIV; PB2-627K host adaptation mutation; CRISPR-Cas system; influenza virus genetics; and reverse genetics for coronavirus. Three of these discoveries best met all of our criteria (Table 10.5) and two, the SARS animal model and the PB2-627K host adaptation mutation, were included in the study. Reverse genetics for coronavirus was excluded because it was too similar to PB2 and another discovery, reconstruction of the 1918 influenza virus (suggested by our own team) was chosen because it met all of our criteria.

[1061] Pfeiffer JK (2015) Is the Debate and "Pause" on Experiments That Alter Pathogens with Pandemic Potential Influencing Future Plans of Graduate Students and Postdoctoral Fellows? *mBio* 6(1): e02525-14.

Table 10.5. Case Studies Suggested in Interviews			
	Discovery year 2000-2005	**Involve SARS, MERS, or influenza virus**	**Expected to lead to GoF research**
SARS animal model (2003)	✓	✓	✓
Growing embryonic stem cells in culture (1998)	N/A	N/A	N/A
HIV chimeric virus macaque animal model (1998)	N/A	N/A	✓
PB2-627K host adaptation mutation in influenza virus (2001)	✓	✓	✓
CRISPR-Cas system (2012)	N/A	N/A	N/A
Reverse genetics for coronavirus (2003)	✓	✓	✓

In summary, the following discoveries were used as case studies:

1. Development of SARS animal model ("SARS-AM"), published in 2003.[1062]
2. Identification of high virulence mutation in H5N1 influenza virus ("Flu-PB2"), published in 2001,[1063] and
3. Reconstruction of 1918 Spanish influenza strain ("Flu-1918"), published in 2005.[1064]

To ensure that these articles represented the starting point for proliferation, we examined all hits to the relevant terms that were generated by PubMed and WoS prior to the publication year. The search yielded several articles and each was examined. We found that all SARS papers were on unrelated topics. In contrast, a few papers involving PB2 gene and 1918 influenza strain appeared relevant based on the abstract. However, closer examination revealed that the PB2 papers implicated the region containing this gene as a contributor to influenza pathogenesis, but did not pinpoint the gene itself.[1065] Similarly, the 1918 influenza papers reported sequencing of various RNA fragments, but not full

[1062] Kuiken T et al (2003) Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet*. 362(9380):263-70

[1063] Hatta M et al (2001) Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science*. 293(5536):1840-2.

[1064] Tumpey TM et al (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science*. 10(5745):77-80.

[1065] O'Neill E et al (2000) Heterologous protection against lethal A/HongKong/156/97 (H5N1) influenza virus infection in C57BL/6 mice. *J Gen Virol*. 81(Pt 11):2689-96

reconstruction.[1066,1067,1068,1069,1070,1071,1072,1073,1074,1075,1076,1077] Based on this analysis, we concluded that the papers by Kuiken et al., Hatta et al., and Tumpey et al. represented the first published reports for the case studies.

Proliferation Trends

Extensive bibliometric searches were conducted to trace the propagation of the discoveries in the research community. After combining and de-duplicating the publication sets we were left with 1,027 articles for SARS-AM, 685 for Flu-PB2, and 479 for Flu-1918. The titles and abstracts of these articles were examined to determine whether they involved infection of animals with relevant strains or manipulation of live virus, and were performed at containment levels of BSL-2+ or higher. After this process, the final set contained 138 SARS-AM, 132 Flu-PB2, and 29 Flu-1918 papers, excluding the three initial discovery articles. Note that at this stage we did not exclude groups working outside of the United States if they published in English.

To characterize the level and rate of proliferation, we examined the number of papers published per year and the number of groups, defined by the last author, publishing these papers. Each group was given one count per year regardless of the number of papers they published in that year, but was counted in each year they published a paper(s). For example, if group A published ten papers in 2005, five papers in 2006, and one paper in 2007, they will receive one count for 2005, 2006, and 2007.

As can be seen from Figure 10.2 (A-C), three discoveries followed different proliferation paths. The SARS animal model was quickly taken up by about 20 groups, but the number of publications began to decline in a few years. The chart shows possible upward trend beginning in 2014, the data are insufficient to make any conclusions. In contrast, the uptake of the PB2 discovery has been slow, but continues to increase. The number of groups working on the 1918 strain has remained low and constant.

1066 (a) Reid AH et al (1999) Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. *Proc Natl Acad Sci U S A*. 96(4):1651-6.

1067 Reid AH et al (2000) Characterization of the 1918 "Spanish" influenza virus matrix gene segment. *Proc Natl Acad Sci U S A* 97(12):6785-90.

1068 Reid AH et al (2002) Characterization of the 1918 "Spanish" influenza virus matrix gene segment. *J Virol*. 76(21):10717-23.

1069 Reid AH et al (2003) Relationship of pre-1918 avian influenza HA and NP sequences to subsequent avian influenza strains. *Avian Dis*. 47(3 Suppl):921-5.

1070 Reid AH et al (2004) Novel origin of the 1918 pandemic influenza virus nucleoprotein gene. *J Virol*. 78(22):12462-70

1071 Basler CF et al (2001) Sequence of the 1918 pandemic influenza virus nonstructural gene (NS) segment and characterization of recombinant viruses bearing the 1918 NS genes. *Proc Natl Acad Sci U S A*. 98(5):2746-51.

1072 Taubenberger JK et al (2001) Integrating historical, clinical and molecular genetic data in order to explain the origin and virulence of the 1918 Spanish influenza virus. *Philos Trans R Soc Lond B Biol Sci*. 356(1416):1829-39.

1073 Gibbs MJ et al (2001) The haemagglutinin gene, but not the neuraminidase gene, of 'Spanish flu' was a recombinant. *Philos Trans R Soc Lond B Biol Sci*. 356(1416):1845-55.

1074 Brownlee GG et al (2001) The predicted antigenicity of the haemagglutinin of the 1918 Spanish influenza pandemic suggests an avian origin. *Philos Trans R Soc Lond B Biol Sci*. 356(1416):1871-6.

1075 Kobasa D et al (2004) Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. *Nature*. 431(7009):703-7

1076 Tumpey TM et al (2004) Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci U S A*. 101(9):3166-71. Epub 2004 Feb 12.

1077 Reid AH et al (2003) 1918 influenza pandemic caused by highly conserved viruses with two receptor-binding variants. *Emerg Infect Dis*. 9(10):1249-53.

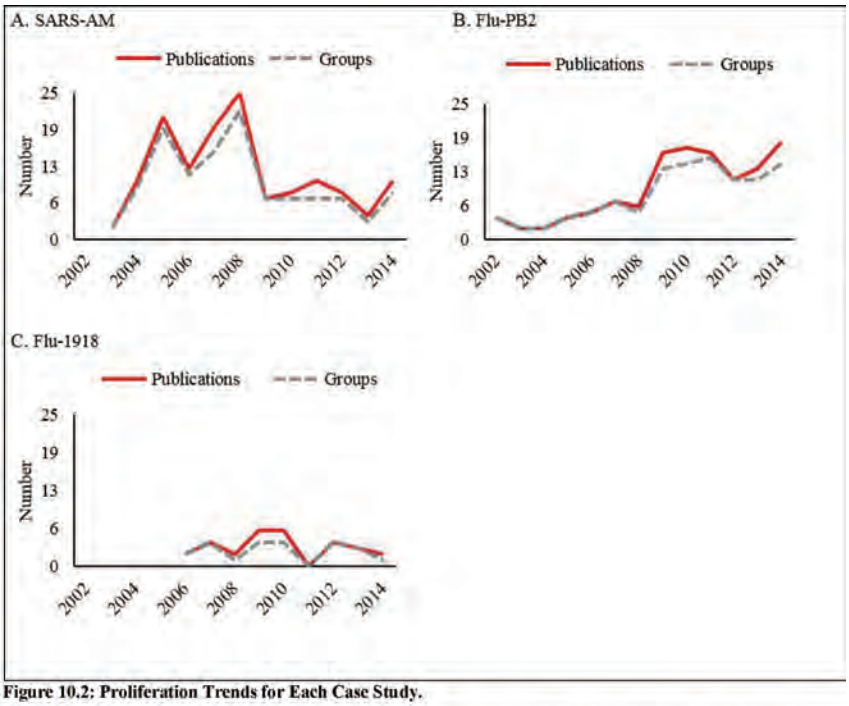

Figure 10.2: Proliferation Trends for Each Case Study.

A number of factors might explain these trends, the most important of which is probably the levels of federal funding, which are in turn dependent on perceived public health needs. In 2003, shortly after the SARS epidemic began, the NIH supported nine intramural projects on SARS research (funding levels were not available from public sources); in 2005 this number increased to 33 ($48M) and in 2008 to 50 ($67M, data not shown). As can be seen from Figure 10.2, the number of groups working on SARS also increased rapidly between 2004 and 2008. However, while funding for SARS remained high (Table 10.3), the number of studies declined in 2009. The reasons for this trend are unclear. It is possible that the researchers in this community encountered similar experimental challenges, which have not yet been resolved. Since there were few, if any new cases since 2004, the sense of public health need may have gradually declined. In 2012, the CDC added SARS to the list of select agents, and while this occurred after the decline took place, increasing containment requirements may be slowing research expansion in the past few years.[1078] The increase in the number of projects in 2014 might reflect the emergence of MERS, another coronavirus, and an associated increase in research interest in SARS. However, at this time we do not have enough data points to determine whether this uptick represents a change in trend.

[1078] Federal Register Vol. 77, No. 194. Friday, October 5, 2012.

In contrast to SARS, influenza outbreaks remain a widely discussed public threat, and this might be one reason for the continued proliferation of the FLU-PB2 discovery. The NIH funding for influenza has also increased dramatically, from $47M in 2000 to $654M in 2014, offering new research opportunities. Finally, the nature of the discovery may have contributed to the trend as well; it appears that many of the papers that followed the initial report tested the PB2 mutation in different influenza strains and animal models. Finally, we found no proliferation of the research reconstructing the 1918 influenza virus. This strain was added to the list of select agents immediately after it was reconstructed in 2005, which probably inhibited proliferation.[1079]

In addition to examining the proliferation trend, we calculated the total number of groups working in each area, since the discovery was made through 2014, which was 85 for SARS-AM, 64 for Flu-PB2 mutation, and 12 for Flu-1918 (Figure 10.3). Excluding all authors on the initial discovery papers produces the estimate of research uptake by new groups: 70 for SARS-AM, 59 for Flu-PB2, and one for Flu-1918. Finally, the number of groups was much lower for SARS and PB2 when all of the papers whose last authors are based outside of the US were excluded. Mean funding levels over 2002–2014 were higher for the established than for new groups, at $29M versus $16M ($p<.05$, data not shown).

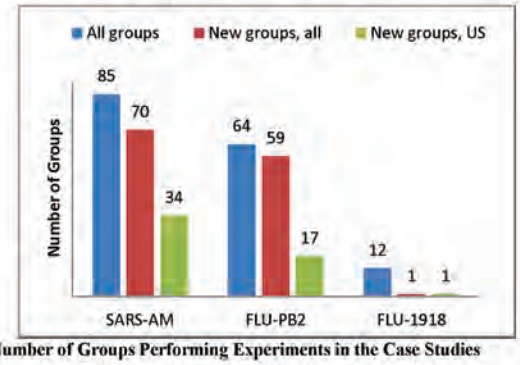

Figure 10.3: Total Number of Groups Performing Experiments in the Case Studies

Table 10.6 shows last authors with at least six publications. Please note that all of the US-based researchers listed in the table have also emerged as members of the interested GOF community (Table 10.3) and as recipients of the NIH funding for influenza and SARS/MERS research (data not shown).

[1079] Federal Register Vol. 70, No. 202 Thursday, October 20, 2005.

Table 10.6. Most Productive Authors in the Case Studies

Author	Number of publications	Case study
Y Kawaoka, University of Wisconsin	19	Flu-PB2, Flu -1918
TM Tumpey, Centers for Disease Control and Prevention	14	Flu -PB2, Flu -1918
RS Baric, University of North Carolina	12	SARS-AM
K Subbarao, National Institute for Allergy and Infectious Diseases	12	SARS-AM, Flu -PB2
HL Chen, Harbin Veterinary Research Institute (China)	9	Flu -PB2
S Perlman, University of Iowa	9	SARS-AM
MG Katze, University of Washington	7	Flu -1918
XF Liu, Yangzhou University (China)	7	Flu -PB2
C Qin, Academy of Sciences (China)	6	Flu -PB2
JK Taubenberger, National Institute for Allergy and Infectious Diseases	6	Flu -PB2, Flu -1918
RG Webster, St Jude Hospital	6	Flu -PB2

Finally, we examined how the discoveries spread through the community. Were they taken up by new researchers with no links to the authors on the initial papers? Or did they propagate via a narrow group of the founders' students and collaborators? To answer these questions, we constructed authorship dendrograms. In one set, we mapped out all last authors who became middle authors on a subsequent paper. We assumed that in these cases the investigator who published earlier provided expertise, strains, or laboratory space and/or other resources to the laboratory that published later. By examining the authorships using this principle, we constructed proliferation dendrograms for each case study (Figures 10.4-10.7).

Not surprisingly, we found that most authors were interconnected. In fact, for the smallest case study of Flu-1918, a single author (Garcia-Sastre, the last author on the index paper) participated in the work of every other last author (Figure 10.4). Similarly, for SARS-AM and Flu-PB2, three authors (Osterhaus/Subbarao/Baric and Kawaoka/Tumpey/Webster, respectively) were key players in the propagation of their research (Figures 10.5 and 10.6). The authors shown in bold and in light grey fonts are independent and non-independent authors at the same institution as their parent, respectively.

Not all authors have left a lasting mark, however. Notice the dots on the bottom of panels B and C; these are the last authors who published a single paper and left no "offspring," at least at present. The fraction of last authors that were connected to other last authors was 100% for Flu-1918, 44% for SARS-AM, and 59% for Flu-PB2. This analysis indicates that a good estimate of the proliferation potential in these fields could be obtained by asking current researchers about their ongoing or planned collaborations and determining if their senior post-docs plan to stay in the same field or move on.

We repeated the analysis by mapping out all middle authors who became last authors on subsequent papers. This group probably represents post-docs and graduate students in the laboratory of a last author would go on to publish similar research as a senior author. While the specific author relationships appeared different, the overall branching pattern held (see Appendix IV). These data suggest that a

discovery moves through the scientific community both through earlier groups giving rise to new groups (which appeared to be the predominant pattern) and through the independent emergence of new groups.

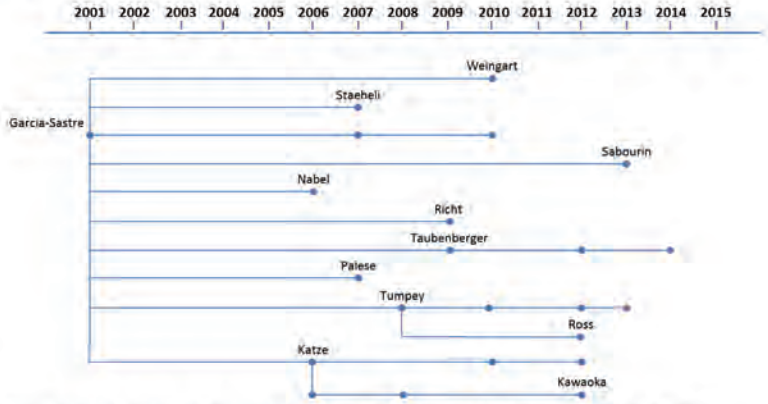

Figure 10.4: Network Diagrams Showing Authorship Relationships for Flu-1918 Case Study.[1080]

[1080] Each dot represents a paper with an indicated last author. If an earlier last author became a middle author on a subsequent paper with a different last author, a line was drawn between the dots. PIs and non-PIs at the same institution are shown in bold font and light gray font.

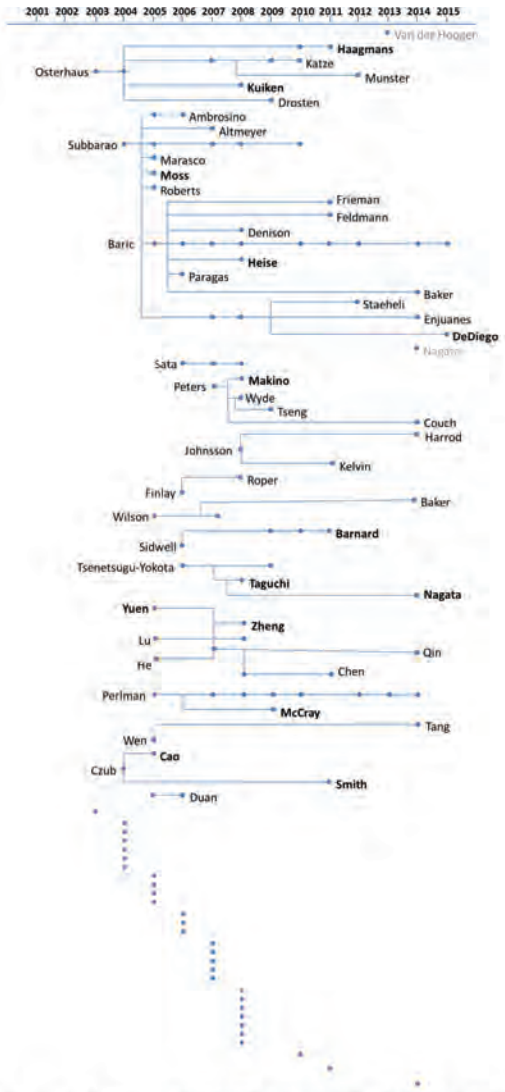

Figure 10.5: Network Diagrams Showing Authorship Relationships for SARS-AM Case Study.

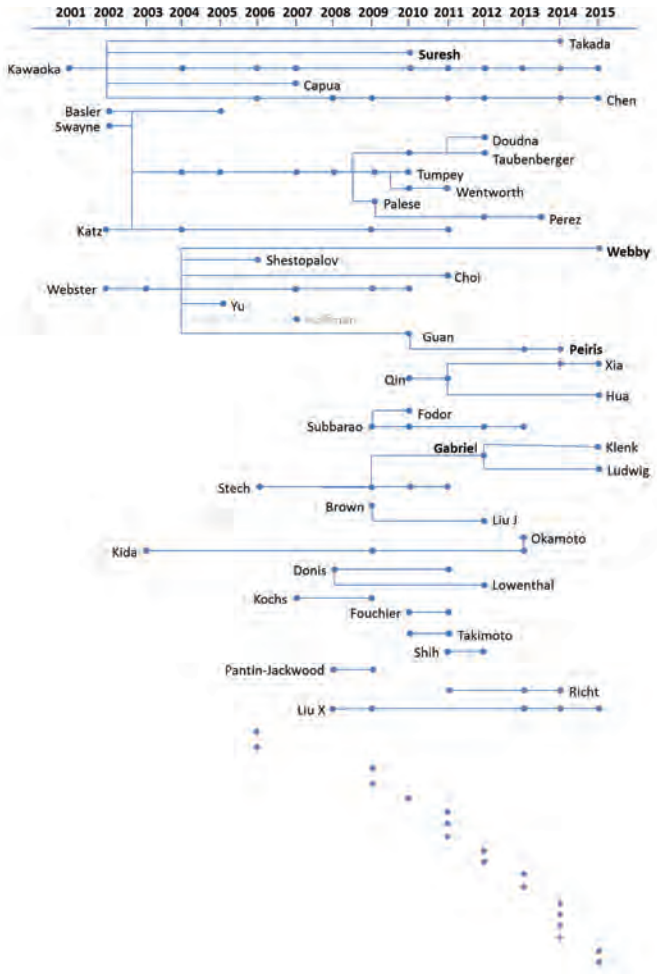

Figure 10.6. Network Diagrams Showing Authorship Relationships for Flu-PB2 Case Study.

Research Sites

We examined the sites in the United States where the GoF experiments published by the groups working on SARS-AM, on Flu-PB2, and on Flu-1918 were performed. As most papers list several affiliations, we made an assumption that the institution of the last author was the experimental site. Figure 10.7 shows the number of institutions for each case study and across the studies; Table 10.7 lists the facilities.

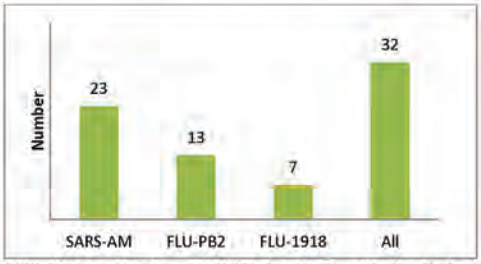

Figure 10.7: Number of Sites Performing Research Related to the Case Studies in the United States by pathogen. An asterisk represents a known BSL-4 facility.

Table 10.7. Sites Performing Research Related to the Case Studies in the United States.	
Battelle Memorial Institute	State University of New York
Baylor University	University of Alabama
Centers for Disease Control and Prevention	University of Iowa
Diagnostic Center for Population and Animal Health	University of Maryland
DynPort Vaccine Company	University of Pittsburgh
East Carolina University	University of Washington
Food and Drug Administration	University of Wisconsin
Harvard University	University of North Carolina
Johns Hopkins University	University of California Berkeley
Kansas State University	University of Rochester
Medical Res Inst for Infectious Diseases, Fort Detrick*	University of Texas Galveston*
Mount Sinai School of Medicine	University of Central Florida
National Institute of Allergy and Infectious Diseases*	US Department of Agriculture
Novavax Inc	Utah State University
Stanford Research Institute	Vanderbilt University
St Jude Children's Hospital	Yale University
**Represents a known BSL-4 facility*	

Finally, we reviewed the papers in the set to identify the biosafety level of the facilities at which the experiments were performed. Figure 10.8 shows that BSL-3 or BSL-3+ was the most common type in all three cases. Note that 21 of 29 papers on 1918 influenza used BSL-3+ or BSL-4 facility, which may explain the paucity of this research, since as far as we could tell the number of these facilities is small.

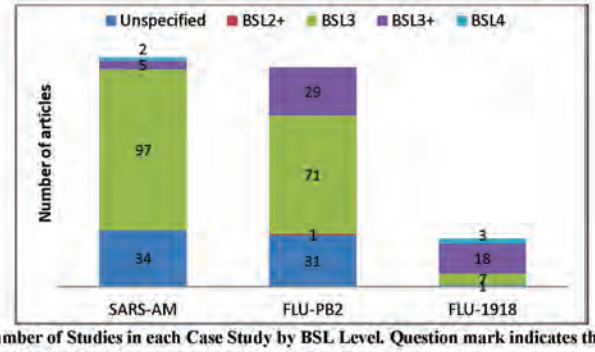

Figure 10.8: Number of Studies in each Case Study by BSL Level. Question mark indicates that the facility level was not specified in the article and assumed to be BSL-3.

NIH Support

As an independent measure of research support, we abstracted the grant numbers that were provided in case study publications dated 2011–2015 and obtained data on the funding amounts for that period. Of the 61 grants and contracts referenced in the papers, funding amounts were available for 32 and totaled $283M over five years, or $57M per year. The median funding level for the 14 PIs was $783K, which was in the same range as what we found in previous analysis (Figure 10.7). Because these estimates are based on the references within the GoF papers, we can conclude that at least some portion of this funding was used on this type of research, but cannot determine the specific amount.

10.6 Conclusions

We identified a group of 40 active, well-funded researchers in the US who have been performing, or have the capacity to perform, certain types of GOF experiments involving influenza, MERS, and SARS viruses. Availability of containment facilities does not appear to be limiting and in the high-proliferation scenario a new discovery in this field may be taken up by as many as 70 new groups around the world within 10-15 years. As indicated by the authorship patterns, about half of the new labs are unconnected to the founders.

While establishing which of the proliferation paths a new discovery will take is impossible *a priori*, some characteristics of the research seem reasonably correlated with greater or lesser proliferation potential. We speculate that broader applicability of the discovery (for example a phenotype-conferring mutation in a common gene or a new method) will facilitate proliferation, and requirements for BSL-3+ or BSL-4 containment facilities will inhibit it, assuming that the number of these facilities does not increase. Negative publicity associated with GoF experiments, additional regulatory oversight, public scrutiny of the research, laboratory accidents, and uncertainty about future funding may limit proliferation.

11 Risk of Loss of Trust in Science

11.1 Summary

The majority of this document examines the risk to public health posed by the misuse of GoF research or an accident at a facility conducting GoF research. However, after an incident of misuse of GoF research or accidents involving a laboratory (e.g., loss of containment), loss of public trust is also a potential and significant outcome. This loss of trust could arise via an accident that caused human illness or deaths, the culling of livestock or wildlife, or even the perception of an increased hazard. Loss of public trust could also arise via the publication of research that is perceived of having little benefit to the public but a great potential for misuse, whether or not this perception is accurate. Overall, without direct polls indicating reasons for loss of trust, assigning responsibility for loss of public trust to specific events is difficult.

The dynamic nature of trust is, however, open to analysis. By examining aspects of different types of trust (e.g., contracts and regulations; the use of standards, certifications, or other assurance; the repetition of positive events or interactions), case studies can be used to understand public loss of trust after specific events originating from the scientific community. While we found no past incident that is a perfect analogy for accidents that could occur during GoF experiments, the case studies on the Tuskegee Syphilis Study and the Fukushima disaster demonstrate how people lost confidence in medical research among the affected minority group or field (nuclear power). For the accidents at Bhopal and Pirbright, while no quantitative sources were found, lawsuits and governmental action reflect a loss of public trust or increase in concern. An increase in governmental regulation or oversight—as was seen in India after Bhopal—reflects a loss of trust in the areas the regulation affects. Regulation and standards also reflect governmental actions aimed at learning from or deriving some benefit from past events—like human subject testing limitations after Tuskegee—in order to prevent them from happening in the future.

Employment and educational data were also examined to provide insight into how events shape the choice of academic and career fields. However, these statistics may not paint an accurate picture due to classification changes in both the academic programs and job categories. This limitation notwithstanding, according to the employment and educational data, a correlation between the events studied and loss of public trust in fields cannot be demonstrated.

From other scientific/ technological incidents examined, the outcomes included an increase in government regulation, lawsuits, and, in the most devastating incidents, a long-term loss in public trust of biomedical research. The longest-lasting harm observed arose from the Tuskegee Syphilis Study, which still reduces African American participation in medical and research studies and affects models of health care delivery to this population to the current day. The other examples presented may perhaps demonstrate a loss of trust, but have seemingly had a minimal effect on research funding, foreign investment, scientific education or scientific employment.

11.2 Purpose and Approach

The concept of trust and its impact on behavior is a widely studied concept, but is generally defined as a dynamic relationship "comprising the intention to accept vulnerability based upon positive expectations of the intentions or behavior of another."[1081] Different forms of trust exist: deterrence-based trust, where methods of control (e.g., contracts, regulations) come into play when sufficient trust is otherwise not present; calculus-based trust, where the presence of control mechanisms is balanced with evidence of a

[1081] Rousseau, D.M., Sitkin, S.B., Burt, R.S., and Camerer, C., Not So Different AfterAll: A Cross-Discipline View of Trust, "Academy of Management, July 1, 1998, 23(3): 393-404. http://amr.aom.org/content/23/3/393.abstract

intentions or credibility (e.g., certifications, 'trust-but-verify'); and relational trust, dependent on repeated, positive interactions or the consistent meeting of expectations.[1082] Thus, the public's trust that the biomedical research establishment can responsibly and safely conduct research on pathogens of pandemic potential can be viewed as a dynamic relationship influenced by the contracts and regulations that are established to control the research, assurances that the research is performed properly and are reinforced by consistently meeting public's expectations for the safety and security of the research. Additionally, in terms of the public's trust of science, loss of trust can be observed at three levels: the institution where the accident occurred, the scientific field involved in the accident, and/or the scientific enterprise in general, which could lead to long term consequences for research and development.

To provide decision-makers with some data on the risk of the loss of public trust in science due to potential incidents involving GoF research, historic incidents were identified related to biomedicine, science or technology and examined available information about the relevant determinants of trust (i.e., contracts and regulations, assurances, and meeting expectations) that reflected the public's loss of trust in scientific institutions, scientific fields, or the scientific enterprise in general.

Case studies were chosen on the basis of available data and applicable "lessons learned." Currently, most information on the topic of loss of public trust is not related to the creation of novel strains of pathogens so it was necessary to analogize from other events. For this assessment, the following incidents and/or accidents were considered:

- 1932-1972, the Tuskegee Syphilis Study in Alabama, USA,
- December 1984, Union Carbide Disaster in Bhopal, India,
- August 2007, Pirbright Foot-and-Mouth Disease Outbreak in Surrey, United Kingdom, and
- March 2011, Fukushima Daiichi nuclear disaster in Okuma, Fukushima, Japan.

Though there have been laboratory releases of agents –smallpox at the University of Birmingham (1966[1083], 1978[1084]), sabia at Yale University (1994[1085]), tularemia at Boston University (2004[1086]), SARS at the National Institute of Virology in Beijing (2004[1087])—there is less information regarding public opinion after these events which may perhaps be related to the small size or effect of these releases compared to the events chosen for the case studies.

For this assessment, a variety of qualitative and quantitative sources regarding each of the above described events were investigated:

- Primary and secondary media reports—including newspapers—provide a snapshot of public reaction after an event, or on the anniversary of an event,
- Scholarly articles from academic journals provide reasoned feedback on the event as well as long-term perspective on the impact of the event,
- Opinion polls measure public reaction quantitatively and provide data on how opinions change over time, and

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[1082] Ibid.
[1083] "Report of the Investigation into the Cause of the 1978 Birmingham Smallpox Occurrence," (July 22, 1980): 30-34
[1084] "Report of the Investigation into the Cause of the 1978 Birmingham Smallpox Occurrence," July 22, 1980.
[1085] "Scientist tests the public trust," *Nature* 371 (September 1, 1994): 1.
[1086] Stephen Smith, "BU delayed reporting possibly lethal exposure," *Boston Globe* (January 20, 2005).
[1087] World Health Organization, "China Confirms SARS infection in another previous reported case; summary of cases to date—Update 5," (April 30, 2004) http://www.who.int/csr/don/2004_04_30/en/

- Lastly, congressional or governmental inquiries and the reports produced reflect public concern in democratic countries.

Beyond the identified events, general studies concerning public trust and confidence in science were gathered and analyzed. Statistics from the US Department of Education's National Center for Education Statistics, Higher Education General Information Survey (HEGIS)[1088,1089,1090] provide the number of university science degrees conferred 1971–2013 and statistics from the National Science Foundation for Science and Engineering Statistics' NSF and NIH Survey of Graduate Students and Post-doctorates in Science and Engineering provides the number of enrolled students for 1975–2011.[1091] The data were considered to identify interruptions of trends that correspond to the dates of historic accidents in order to evaluate if accidents affect student completion of scientific degrees (BS, MS, and PhD). Employment data comes from the US Bureau of Labor and Statistics' Occupational Outlook Handbook between 1972 and 2004.[1092] Employment numbers can be considered estimates of the professionals working in a given field, to determine if the historical events influenced overall employment in the field related to the incident.

11.3 Results

Overall, the data suggests that after an accident or disaster, the public is able to identify the responsible party (for example, after the Fukushima Daiichi disaster, the most loss of public trust was suffered by nuclear power) rather than blaming science generally—this is elaborated upon in each event specific section, below. For each of the events outlined in this report, governmental action (e.g., governmental inquiries or amendments to or the creation of legislation, lawsuits brought against the government, or payment to affected individuals) reflected public concern but was set forth, largely, to prevent similar events in the future. Although some incidents have led to measurable outcomes suggesting a loss of public trust, an investigation of trends in enrollment and hiring in applicable STEM fields identified no negative impacts following an event, which can be directly attributed to that event.

11.3.1 The Tuskegee Syphilis Study: Loss of Trust in Medical Research among African Americans

The US Public Health Service conducted an observational study of untreated syphilis in rural African American men in Alabama from 1932-1972. The investigators did not fully disclose the nature of the study to the participants, falsely told participants they were receiving treatment and did not offer or provide medical interventions/treatments when they became available. The victims of the study included 28 men who died as a direct result of syphilis, 100 men who died of complications related to syphilis, 40 wives of participants, and 19 children born with congenital syphilis.

1088 U.S. Department of Education, National center for Education Statistics, "Table 322.10. Bachelor's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2011-12," https://nces.ed.gov/programs/digest/d13/tables/dt13_322.10.asp

1089 U.S. Department of Education, National Center for Education Statistics, "Table 323.10. Master's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2012-13," https://nces.ed.gov/programs/digest/d14/tables/dt14_323.10.asp

1090 U.S. Department of Education, National Center for Education Statistics, "Table 324.10. Doctor's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2012-13," https://nces.ed.gov/programs/digest/d14/tables/dt14_324.10.asp

1091 National Science Foundation "Survey of Graduate Students and Postdoctorates in Science and Engineering" http://www.nsf.gov/statistics/srvygradpostdoc/

1092 U.S. Department of Labor, Bureau of Labor Statistics "Occupational Outlook Handbook," Washington DC, http://search.lib.virginia.edu/catalog/000046071

The public learned of the Tuskegee Syphilis Study after a 1972 article in the *Washington Star* exposed the study.[1093] Prior to the 1972 article, two young physicians separately wrote to the PHS on three occasions with ethical concerns regarding the study.[1094,1095] One letter led the CDC to convene a blue ribbon panel in 1969 that considered the study, recommending that the study be upgraded "scientifically," yet decided against treating the participants.[1096] Of note, prior to this panel the US Government led in the creation of the Nuremberg Code (1942) to protect the rights of research subjects and generally recognized the Declaration of Helsinki (1964), however this recognition of the ethical need for human subjects' protection was not reflected in the continued consideration or conduct of the Tuskegee Syphilis Study. Since the 1972 article exposing the study,[1097] the US government has attempted to make amends with the public in a variety of ways. The NAACP filed a class action lawsuit on behalf of the study participants seeking $3 million in damages for every living participant and the heirs of each participant. In December 1974, the US Government settled out of court agreeing to pay $37,000 in damages to each survivor—along with lifetime medical benefits for the survivors and any affected family members—and $15,000 for the heirs of deceased study participants.[1098] The aftermath of the Tuskegee Syphilis Study also led to lasting changes in the conduct of research involving human subjects. In 1974, Congress passed and enacted the National Research Act, which created the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. Despite monetary compensation and legislative enactments, the US government did not formally apologize for the Tuskegee Syphilis Study until 1997.

The legacy of the Tuskegee Syphilis Study is also thought by many to extend to the continued lack of trust between the African American community and the US medical system. In various studies of public opinion since the closure of the Tuskegee study, African Americans point to these experiments as proof that the medical research establishment and/or the US government cannot be trusted in terms of equal health care for all races or for providing informed consent: "The continuing legacy of the Tuskegee Syphilis Study has contributed to Blacks' belief that… public health authorities cannot be trusted."[1099,1100,1101,1102,1103,1104] This distrust has been described by scholars dealing with the hesitance of African

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1093 Abigail Perkiss, "Public Accountability and the Tuskegee Syphilis Experiments: A Restorative Justice Approach," *Journal of African American Law and Policy* 70 (2008): 71.

1094 Sarah Kaplan, "Dr. Irwin Schatz, the first, lonely voice against infamous Tuskegee study, dies at 83," The Washington Post, April 20, 2015, https://www.washingtonpost.com/news/morning-mix/wp/2015/04/20/dr-irwin-schatz-the-first-lonely-voice-against-infamous-tuskegee-study-dies-at-83/

1095 Stephen B. Thomas, PhD, and Sandra Crouse Quinn, Med, "The Tuskegee Syphilis Study, 1932 to 1972: Implications for HIV Education and AIDS Risk Education Programs in the Black Community," *American Journal of Public Health* Vol 81, No. 11 (1991): 1499, http://minority-health.pitt.edu/393/1/The_Tuskegee_Syphilis_Study_1932_to.pdf.

1096 Ibid.

1097 Abigail Perkiss, "Public Accountability and the Tuskegee Syphilis Experiments: A Restorative Justice Approach," *Journal of African American Law and Policy* 70 (2008).

1098 Ibid.

1099 Giselle Corbie-Smith, MD, Stephen B. Thomas, PhD, Mark V. Williams, MD, and Sandra Moody-Ayers, MD, "Attitudes and beliefs of African Americans Toward Participation in Medical Research," *Journal of General Internal Medicine* 14 (1999).

1100 Vicki S. Freimuth, Sandra Crouse Quinn, Stephen B. Thomas, Galen Cole, Erik Zook, and Ted Duncan, "African Americans' Views on Research and the Tuskegee Syphilis Study," *Social Science and Medicine* 52 (2001): 797-808.

1101 Benjamin R. Bates, PhD, and Tina M. Harris, PhD, "The Tuskegee Study of Untreated Syphilis and Public Perceptions of Biomedical Research: A Focus Group Study," *Journal of the National Medical Association* Vol. 96, No. 8 (August 2004): 1051-1064.

1102 Bernard Lee Green, Richard Maisiak, Min Qi Wang, Marcia F. Britt, and Nonie Ebeling, "Participation in Health Education, Health Promotion, and Health Research by African Americans: Effects of the Tuskegee Syphilis Experiment," *Journal of Health Education* 28 (1997).

1103 Vickie L. Shavers, PhD, Charles F. Lynch, and Leon F. Burmeister, "Knowledge of the Tuskegee Study and its Impact on the Willingness to Participate in Medical Research Studies," *Journal of the National Medical Association* 92 (2000).

1104 Stephen B. Thomas, PhD, and Sandra Crouse Quinn, Med, "The Tuskegee Syphilis Study, 1932 to 1972: Implications for HIV Education and AIDS Risk Education Programs in the Black Community," *American Journal of Public Health* Vol 81, No. 11 (1991): 1499, http://minority-health.pitt.edu/393/1/The_Tuskegee_Syphilis_Study_1932_to.pdf.

Americans to participate in medical research generally and HIV/AIDS research specifically.[1105,1106,1107] In her study of the Tuskegee Syphilis Study, Abigail Perkiss says, the "United States government had committed gross injustices against members of the African-American community, and that community as a whole was now beset by rampant distrust and suspicion toward the government and the medical profession."[1108] This loss of trust in the biomedical research enterprise in general illustrates how loss of trust from a particular incident may harm trust in biomedical research in general, despite institutionalized changes to address the cause of the incident.

11.3.2 Bhopal Chemical Disaster

Arguably the worst industrial accident in history occurred in Bhopal, India where Union Carbide built and operated a pesticide manufacturing plant. On December 3, 1984, more than 40 tons of methyl isocyanate gas was released into the atmosphere, killing nearly 4,000[1109] people instantly and harming the health of an additional 15,000[1110] to 600,000[1111] people from acute and long term effects.

Investigations into the causes of the disaster found evidence of violations to operating procedures, as well as a damning report from a 1982 safety inspection conducted by representatives from the US-based Union Carbide.[1112] This report indicated serious safety problems at the Bhopal plant and recommended replacing one of the plants main safety devices (water spray system). In addition, research into the causes of the disaster describe a state of confusion over ultimate responsibility for the plant's and the public's safety, questions of legal accountability, and a poor safety culture and low morale among staff at the plant.

Nearly immediately after the chemical release, those affected sought legal recourse both in American and Indian courts of law. The cases brought in the US were dismissed with the commentary that Indian courts could better deal with these issues. However, neither Union Carbide nor DOW Chemical (the owner of what was formerly Union Carbide) have ever formally taken responsibility for the accident at Bhopal and have repeatedly placed the blame on the Indian staff at the plant. No one at DOW has been held criminally liable, though in 2010 eight Indian mid-level managers were convicted of criminal negligence. The government of India enacted the Bhopal Gas Leak Disaster Act as a way of "ensuring that claims arising from the disaster would be dealt with speedily and equitably."[1113] The eventual legal settlement in India was $470 million which would be paid to claimants as part of a full and final settlement. In 2003, the Bhopal Gas Tragedy Relief and Rehabilitation Department reported that monetary relief had been awarded to 554,895 people for injuries sustained in the disaster and to survivors of 15,310 who were

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[1105] Sengupta S, et. al. (2000) "Factors Affecting African-American Participation in AIDS Research," *Journal of Acquired Immune Deficiency Syndromes* 24: 275-284.

[1106] Thomas S, Quinn S (1991) "The Tuskegee Syphilis Study, 1932 to 1972: Implications for HIV Education and AIDS Risk Education Programs in the Black Community," *American Journal of Public Health* Vol 81, No. 11

[1107] Hagen K (2005) "Bad Blood: The Tuskegee Syphilis Study and Legacy Recruitment for Experimental AIDS Vaccines," *New Directions for Adult and Continuing Education* 105

[1108] Perkiss A (2008) "Public Accountability and the Tuskegee Syphilis Experiments: A Restorative Justice Approach," *Journal of African American Law and Policy* 70: 72-73.

[1109] Broughton E (2005) "The Bhopal disaster and its aftermath: a review," *Environmental Health: A Global Access Science Source* 4.

[1110] Ibid.

[1111] Malik A (2014) "30 Years After the Bhopal Disaster, India had not Learned the Lessons of the World's Worst Industrial Tragedy," *International Business Times*.

[1112] Diamond S (1985) The Phopal Disaster: How it Happened, The New York Times. http://www.nytimes.com/1985/01/28/world/the-bhopal-disaster-how-it-happened.html?pagewanted=all .

[1113] Broughton E (2005) "The Bhopal disaster and its aftermath: a review," *Environmental Health: A Global Access Science Source* 4.

killed. The average award amount was $2,200 for families of those killed[1114] and $400 for those who survived.[1115]

Beyond financial compensation, the governments of the United States and India passed legislation in reaction to Bhopal. In 1990 the United States Congress passed the Clean Air Act Amendments (CAAA) sections of this legislation require factories and other businesses to develop plans to prevent accidental releases of highly toxic chemicals. The CAAA also established the Chemical Safety Board, an independent agency that investigates and reports on accidental releases of toxic chemicals from industrial factories.[1116] In India, multiple new pieces of legislation were passed in response to Bhopal including the Environment Protection Act of 1986, amendments to the Indian Factories Act[1117] and the Air Act in 1987, Hazardous Water Management and Handling Rules in 1989, and the Public Liability Insurance Act of 1991.[1118] Together, these pieces of legislation provide a framework similar to what exists in the US and enable the Indian government to react to and prevent future accidents like the one at Bhopal as well as setting forth best practices for handling hazardous waste or running industrial factories.

The influence of the Bhopal disaster on governmental decision making in terms of increased regulation of chemical plants or foreign companies operating within India could be reflected in the levels of foreign direct investment (FDI) before and after the Bhopal Chemical Disaster; if the Indian government enacted legislation making India less attractive to foreign businesses after Bhopal, a decrease in the rate of growth for overall FDI investment levels could be expected.[1119] Overall, while no obvious dip was observed in total FDI in the years following the Bhopal disaster (not shown), we note that prior to 1985 investment in the chemical and pharmaceutical industries was an increasing share of the country's total FDI (blue line in Figure 11.1). In contrast, the data available for 1987 (after the Bhopal Disaster) marks the beginning of a decline in the chemicals and pharmaceuticals sector's share of total FDI relative to others. The rapid growth of FDI in India's chemicals and pharmaceuticals sector during this time period appears to have settled a bit prior to the 1884 Bhopal incident, however the 1987 data indicate a distinct dip in the percent increase in the sector's FDI. That being said, although this sector captured a somewhat smaller share of FDI after Bhopal, the sector still experienced a 100% annual growth rate after Bhopal (red line in Figure 11.1). Although these data represent merely a correlation with any incident, the reaction to the events at Bhopal may have resulted in a decrease in foreign investment in India's chemical and pharmaceutical sectors.

[1114] Ibid.

[1115] Malik A (2014) "30 Years After the Bhopal Disaster, India had not Learned the Lessons of the World's Worst Industrial Tragedy," *International Business Times*.

[1116] United States Environmental Protection Agency, "The Plain English Guide to the Clean Air Act" (April 2007): 17.

[1117] "The Factories Act, 1948 (Act No. 63 of 1948), as amended by the Factories (Amendment) Act 1987 (Act 20 of 1987)" https://www.ilo.org/dyn/natlex/docs/WEBTEXT/32063/64873/E87IND01.htm

[1118] Mannan M, et. al. (2005) "The legacy of Bhopal: The impact over the last 20 years and future direction," *Journal of Loss Prevention in the Process Industries* 18: 221.

[1119] http://data.worldbank.org/indicator/BX.KLT.DINV.CD.WD/countries

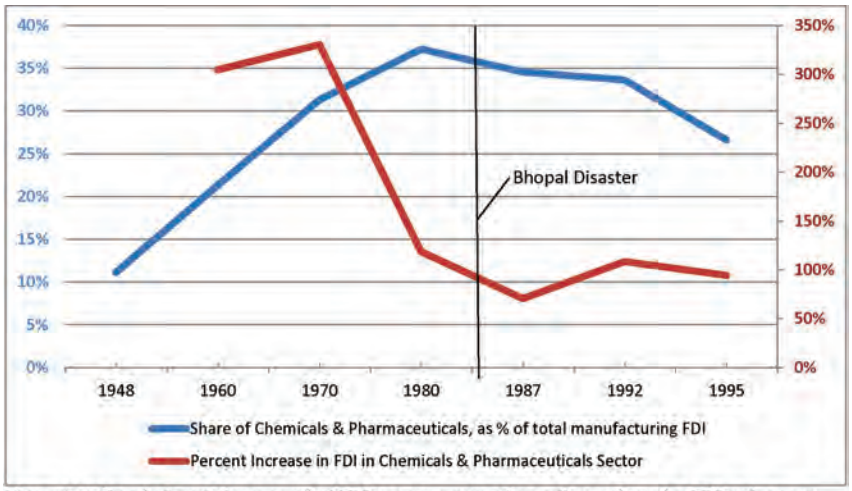

Figure 11.1 Chemicals and pharmaceutical FDI as a percentage of overall manufacturing FDI and percent increase in FDI in the chemicals and pharmaceuticals sector. [1120] Approximate timing of the Bhopal Chemical Disaster is shown.

11.3.3 Pirbright FMD Outbreak

In August 2007, there were multiple outbreaks of Foot and Mouth Disease (FMD) among cattle herds in Surrey, England. Well aware of the impact of FMD from the 2001 outbreak, farmers were forced to cull their animals quickly to stem the spread of the disease. Overall, as the outbreaks began, the affected public identified many possible environmental sources—migrating geese, local deer, or dogs.[1121] Analysis of the virus indicated that it was a strain of FMD isolated from the 1967 outbreak and used as a vaccine strain by the nearby Pirbright Institute, which housed both the Institute for Animal Health (IAH, a government diagnostic, research, and international reference lab run by the Department for Environment, Food & Rural Affairs) and Merial Animal Health Ltd (a vaccine manufacturing factory). The consensus of numerous governmental inquiries and commissions[1122,1123,1124,1125] was that the pathogen was likely accidentally released from the decades-old Pirbright facilities[1126] through leaking effluent pipes and a

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[1120] Adapted from Suma Athreye and Sandeep Kapur, "Private Foreign Investment in India," August 1999, http://www.bbk.ac.uk/ems/faculty/kapur/personal/fdi.pdf

[1121] Gray R (2007) "National Trust Estate Hit by Foot and Mouth," *The Telegraph*.

[1122] Return to an Address of the Honourable House of Commons, "Foot and Mouth Disease 2007: A Review and Lessons Learned" March 11, 2008.

[1123] Department of Environment, Food, and Rural Affairs (2007) "A Review of the Regulatory Framework for Handling Animal Pathogens".

[1124] House of Commons, Innovation, University, Science, and Skills Committee, "Biosecurity in UK Research Laboratories," June 28, 2008.

[1125] Health and Safety Executive, "Final Report on Potential Breaches of Biosecurity at the Pribright site 2007," December 20, 2007.

[1126] Department of Environment, Food, and Rural Affairs (2007) "A Review of the Regulatory Framework for Handling Animal Pathogens,": iii.

faulty valve at the Merial vaccine manufacturing plant.[1127] That the pathogen was accidentally—as opposed to intentionally—released may offer the closest example of scientific accident to one that could occur while conducting GoF research in the United States.

Farmers who were affected by the depopulation of livestock brought a £1.5 million lawsuit against the Institute for Animal Health and Merial Animal Health, as well as the Secretary of DEFRA.[1128] IAH and Merial settled with half of the farmers, while admitting no liability, and a judge dismissed the claims of the other half (since none of their animals had been culled).[1129]

IAH at Pirbright is a critical facility for work with dangerous animal pathogens in the UK, and prior to the 2007 FMD release, the labs were due to be updated. The FMD accident did not prevent the renovation of the research facility for the IAH which was approved in July 2009[1130] at a cost of £137 million. However, as general budget discussions and austerity measures, were implemented in the UK, the funding for Pirbright and three other priority government funded science projects were cancelled.[1131] Under these austerity measures, funding was cut throughout the government—for health, business, local governments, etc.— and not just for science.[1132] Funding for the redevelopment of Pirbright was reorganized and covered between the Biotechnology and Biological Sciences Research Council (BBSRC), DEFRA, and the Department for Innovation Universities and Skills. At the time of this writing, the renovations are still ongoing at Pirbright, however the new biocontainment facilities are already in use.[1133] In short, no data was found conclusively tying any negative consequences to science from this incident.

11.3.4 Fukushima Daiichi: Re-evaluation of Nuclear Power Worldwide

In March 2011, the massive Great East Japan Earthquake triggered a tsunami that disrupted cooling systems at the Fukushima Daiichi Nuclear Power Plant resulting in several core meltdowns and damage to the spent fuel. Radioactive material was released from the three affected reactors and people living within a 30km radius of the plant were evacuated.[1134] The financial cost of Japan's recovery from the Fukushima disaster is still ongoing, with nearly 250,000 Japanese still displaced[1135] and the country importing 90% of its energy.[1136]

Retrospective analyses of the factors that led to the Fukushima accident abound, including an influential report from the Fukushima Nuclear Accident Independent Investigation Commission (NAIIC), an independent body created by the National Diet (Japan's parliament). The findings of the report point to "a multitude of errors and willful negligence that left the Fukushima plant unprepared for the events of March 11," as well as "serious deficiencies in the response to the accident by TEPCO, regulators and the

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1127 Return to an Address of the Honourable House of Commons. "Food and Mouth Disease 2007: A Review and Lessons Learned," March 11, 2008: 12.

1128 Balakrishnan A (2008) "Farmers sue for damages in Pirbright foot-and-mouth outbreak," *The Guardian*. http://www.theguardian.com/uk/2008/oct/17/footandmouth-ruralaffairs

1129 "Foot-and-mouth cash demand fails," BBC, March 31, 2009, http://news.bbc.co.uk/2/hi/uk_news/7974982.stm

1130 "Spending Review: Pirbright research lab escapes cuts," BBC, October 20, 2010, http://www.bbc.com/news/uk-england-surrey-11588361

1131 Sample I (2011) "Research cuts will force scientists to share laboratories, top academics warn," *The Guardian*, http://www.theguardian.com/science/2011/may/11/cuts-endanger-science-research-teaching

1132 "Spending Review 2010: Key points at-a-glance," BBC, October 21, 2010, http://www.bbc.com/news/uk-politics-11569160

1133 http://www.research.pirbright.ac.uk/redevelopment/ "Phase two…is expected to be complete around 2016."

1134 Siegrist M, Visschers V (2013) "Acceptance of Nuclear Power: The Fukushima Effect," *Energy Policy* 59: 112.

1135 Spitzer K (2015) "250,000 japanese still displaced 4 years after quake, USA Today, http://www.usatoday.com/story/news/world/2015/03/09/japan-tsunami-radiation-fourth-anniversary-fukushima/24254887/

1136 Fukushima's impact on Japans economy three years on, BBC News, March 11, 2014, http://www.bbc.com/news/business-26524084 .

government."[1137] More specifically, TEPCO and NISA were both cited for inadequately assessing the earthquake and tsunami hazards faced by the plant. For example, TEPCO's modeling did not adequately incorporate the IAEA-promulgated best practice of including historic and pre-historic (i.e., evidence from Japan's geological record) seismic events and tsunamis. The committee also noted that a 2008 study by TEPCO itself suggested that the tsunami hazard was greatly underestimated, however the company never followed-up on this finding. As a result of the failure to take historical event into account meant that the Fukushima plant was not designed to withstand a tsunami of even half the magnitude of the March 2011 event.[1138] NAIIC Chairman Kiyoshi Kurokawa stated that "nuclear power became an unstoppable force" in Japan which was "immune to scrutiny by civil society." He continues that "Japan's nuclear industry managed to avoid absorbing the critical lessons learned from Three Mile Island and Chernobyl."[1139]

The long-term effects of the Fukushima Daiichi nuclear disaster reached far beyond Japan's borders. In the days after the disaster, and with shaken confidence in nuclear power, governments around the world performed tests and checks on their own reactors, took reactors offline, or started dialogues about the future of nuclear power in their country. The European Union called for voluntary stress tests on reactors within the EU and member countries reacted in various ways. Germany, with 17 reactors[1140], shut down the seven oldest, pending safety tests; Britain, with 19 reactors, and France, with 58 reactors, planned safety reviews but decided not to delay nuclear expansion plans; Poland and the Czech Republic were unaffected by the disaster and planned to continue with their nuclear plan development. Outside of Europe, China temporarily suspended work on the approximately two dozen reactors under construction, planned checks for operating reactors, and considered changes to their long-term nuclear power expansion plans. Other earthquake prone countries, like India and Turkey, continued their nuclear development plans unaffected by the Fukushima disaster.[1141]

Though caused by a "natural" event, the reaction of Japan's public reflected a "radical alteration of … a[n] optimistic view on science in policy making,"[1142] a loss of public trust in both the impartiality of scientists, of science in general.[1143] Farther afield, the Pew Research Center,[1144] conducted telephone interviews in the United States immediately after the Fukushima accident to assess opinions on nuclear power issues. In March 2011 39% of those polled favored promotion of increased nuclear power use while 52% opposed. As a comparison, in October 2010, 45% favored and 44% opposed, demonstrating that even though the accident did not occur in the US, 6-8% of Americans views of nuclear power became more negative. Ipsos Global @dvisor, likewise, conducted a survey in May 2011 in 24 countries. Ipsos found that countries in South and Southeast Asia—including South Korea, Japan, China, and India—identified recent events in Japan as the source of their opposition to nuclear power.[1145]

Financial remuneration was offered to those living in Fukushima who were affected by the disaster. The total bill for clean-up and remuneration of displaced residents is currently estimated at $137 billion (USD)

[1137] The National Diet of Japan "The Official Report of the Fukushima Nuclear Accident Independent Investigation Commission," 2012: 9.
[1138] Carnegie endowment report.
[1139] The National Diet of Japan "The Official Report of the Fukushima Nuclear Accident Independent Investigation Commission," 2012: 9.
[1140] Kim Y, Kim M, Kim W (2013) "Effect of the Fukushima nuclear disaster on global public acceptance of nuclear energy," *Energy Policy* 61: 822-823.
[1141] "Fukushima fall-out for reactors around the world" *Nature.com*, March 21, 2011. http://blogs.nature.com.mutex.gmu.edu/news/2011/03/fukushima_fallout_for_reactors.html.
[1142] Arimoto T, Sato Y (2012) "Rebuilding Public Trust in Science for Policy-Making," *Science* 337: 1176.
[1143] Ibid.
[1144] Pew Research Center, "Opposition to Nuclear Power Rises Amid Japanese Crisis" March 21, 2011, 2-3.
[1145] Ipsos—Global @dvisor, "Global Citizen reaction to the Fukushima Nuclear Plant Disaster" June 2011, 5.

total, with costs to be covered by government issued bonds that TEPCO will repay over time.[1146,1147] While the event has caused a drag on the Japanese economy and affected public opinion on the safety of nuclear technology worldwide, the lasting impact of Fukushima may be to highlight the need to revise risk calculations, and the resulting safety margins, with current knowledge. As stated by James Acton and Mark Hibbs for the Carnegie Endowment for International Peace, "In the final analysis, the Fukushima accident does not reveal a previously unknown fatal flaw associated with nuclear power. Rather, it underscores the importance of periodically reevaluating plant safety in light of dynamic external threats and of evolving best practices, as well as the need for an effective regulator to oversee this process."[1148]

11.3.5 Effect of Incidents on US Scientific Education

It was hypothesized that if any of these significant events harmed the US public's perception of science, fewer students would be attracted to, enroll in, and later complete, degrees in related fields. The US was the focus of this study because the relevant data was available in English and the current study examines the effect of US action on risk of GoF research. It is recognized that a student in a degree program may not drop the program due to a scientific accident, so the numbers may not drop immediately after an event (if they drop at all). To control for economic factors that may influence the overall enrollment in post-secondary education, the focus was on the percent of students that enroll or complete a degree compared to all those enrolling in post-secondary education. National data showing the percentage of scientific degrees earned (Figure 11.2 and 11.3) from the total number of degrees earned show no dips that could be attributable to any particular incident/accident—physical sciences degrees have remained steady and biological and biomedical degrees increased around the mid-2000s. The dip observed in Ph.D. completion after the Tuskegee experiments occurs too soon after the revelation of the experiments to be attributable to this event. Of note, an increase in the completion of undergraduate degrees in the life sciences is seen four years after the 1972 revelation of the experiments. This uptick in life sciences undergraduate degrees earned takes place two years after the influential 1975 Asilomar Conference on Recombinant DNA which increased public interest in genetics and biomedical research.[1149] Perhaps the decline in physical science degrees since the post-Sputnik surge is partially attributable to the Bhopal disaster, but the downward trend in physics Ph.D.s granted by US institutions began before the incident.[1150] Chemical engineering has remained strong[1151] and well represented in the engineering field (Figure 11.4), while nuclear and biomedical remain steady with relatively low enrollment, non-respective of scientific accidents.

[1146] Inajima T, Song Y (2012) $137 Billion Cost has Tepco Seeking more Aid, *Bloomberg Business*. http://www.bloomberg.com/news/articles/2012-11-07/fukushima-137-billion-cost-has-tepco-seeking-more-aid.

[1147] Also, Catherine Butler, Karen A. Parkhill, and Nicholar F. Pidgeon, "Nuclear Power After Japan: The Social Dimensions. *Environment: Science and Policy for Sustainable Development* 53:6 (2011): 6.

[1148] Carnegie endowment report.

[1149] Berg P, Singer M (1995) "The recombinant DNA controversy: Twenty years later", *PNAS*, http://www.pnas.org/content/92/20/9011.

[1150] Kaiser D (*American Physics and the Cold War Bubble*, (University of Chicago Press, in preparation). http://web.mit.edu/dikaiser/www/CWB.html .

[1151] It is unclear what the large drop between 2002 and 2003 can be attributed to. Based on the way these studies are conducted, it is likely attributed to a reclassification of the degree or program type, however, no such information explaining this was found within the National Science Foundation's records.

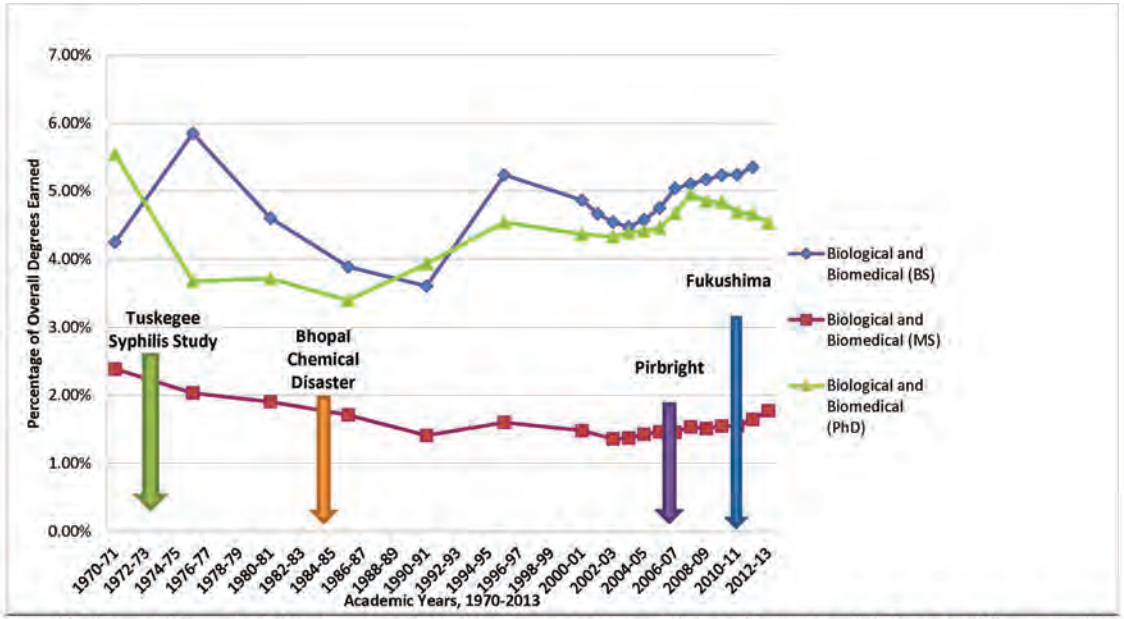

Figure 11.2. Percent of degrees in the life sciences awarded by US Institutions as a percent of the total degrees awarded. The timing of the catastrophic events is shown. Statistics drawn from the US Department of Education's National Center for Education Statistics, Higher Education General Information Survey (HEGIS).[1152,1153,1154]

[1152] U.S. Department of Education, National center for Education Statistics, "Table 322.10. Bachelor's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2011-12," https://nces.ed.gov/programs/digest/d13/tables/dt13_322.10.asp

[1153] U.S. Department of Education, National Center for Education Statistics, "Table 323.10. Master's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2012-13," https://nces.ed.gov/programs/digest/d14/tables/dt14_323.10.asp

[1154] U.S. Department of Education, National Center for Education Statistics, "Table 324.10. Doctor's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2012-13," https://nces.ed.gov/programs/digest/d14/tables/dt14_324.10.asp

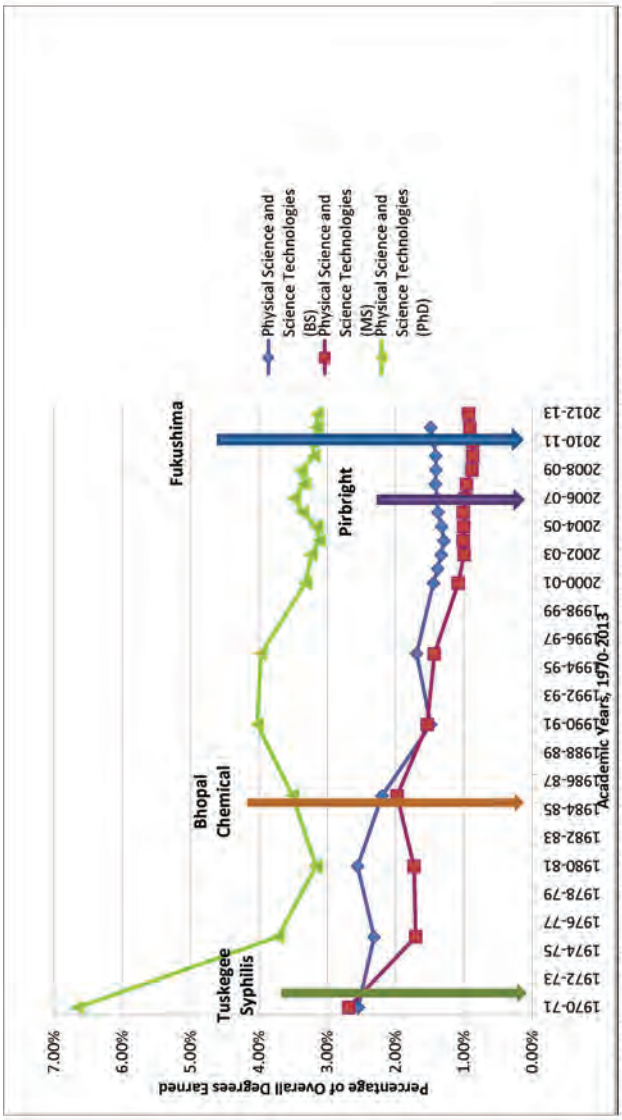

Figure 11.3. Percent of degrees in the physical sciences awarded by US institutions as a percent of the total degrees awarded. The timing of the catastrophic events is shown. Statistics from the US Department of Education's National Center for Education Statistics, Higher Education General Information Survey (HEGIS).[1155,1156,1157]

1155 U.S. Department of Education, National center for Education Statistics. "Table 322.10. Bachelor's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2011-12," https://nces.ed.gov/programs/digest/d13/tables/dt13_322.10.asp

1156 U.S. Department of Education, National Center for Education Statistics. "Table 323.10. Master's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2012-13," https://nces.ed.gov/programs/digest/d14/tables/dt14_323.10.asp

1157 U.S. Department of Education, National Center for Education Statistics. "Table 324.10. Doctor's degrees conferred by postsecondary institutions, by field of study: Selected years, 1970-71 through 2012-13," https://nces.ed.gov/programs/digest/d14/tables/dt14_324.10.asp

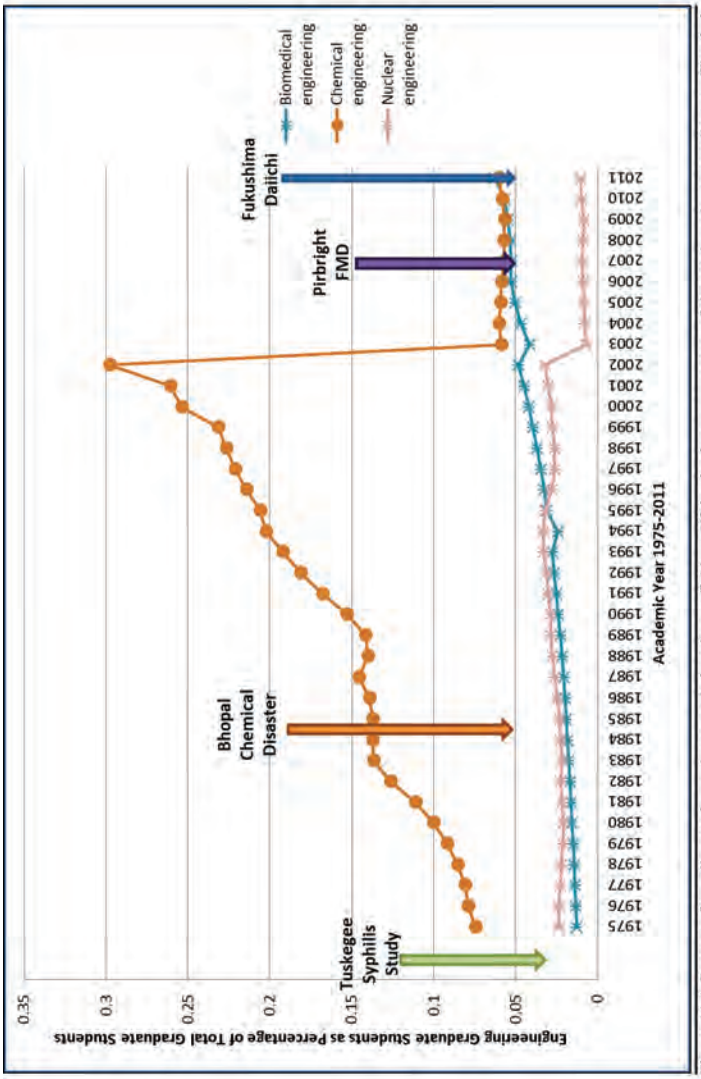

Figure 11.4. Percent of enrollments in graduate study in relevant fields at US institutions as a percent of the total graduate enrollments. The timing of the catastrophic events is shown. Statistics from the National Science Foundation for Science and Engineering Statistics' NSF and NIH Survey of Graduate Students and Post-doctorates in Science and Engineering.[1158]

[1158] National Science Foundation "Survey of Graduate Students and Postdoctorates in Science and Engineering" http://www.nsf.gov/statistics/srvygradpostdoc/

11.3.6 Effect of Incidents on Scientific Employment

Similar to the rationale described above, if any of the described events harmed public perception of science, that data may show a drop in number of employees in relevant fields. In the case of employment data, it is possible that an event would cause an immediate drop in employment if workers left their jobs in disgust. The number employed in a variety of related fields (Figure 11.5) does not appear to be affected by any event described in this report, though it may reflect economic factors not related to any particular scientific accident or event. Moreover, any drop in employment could have been compensated for by the filling of vacancies with employees on a work visa. Another possible explanation for drops in fields could be shifting categories of employment (for example, Biological and Biomedical fields are combined in some years and separate for others). In 1998, shifts upwards and downwards are likely a result of these changing category designations.

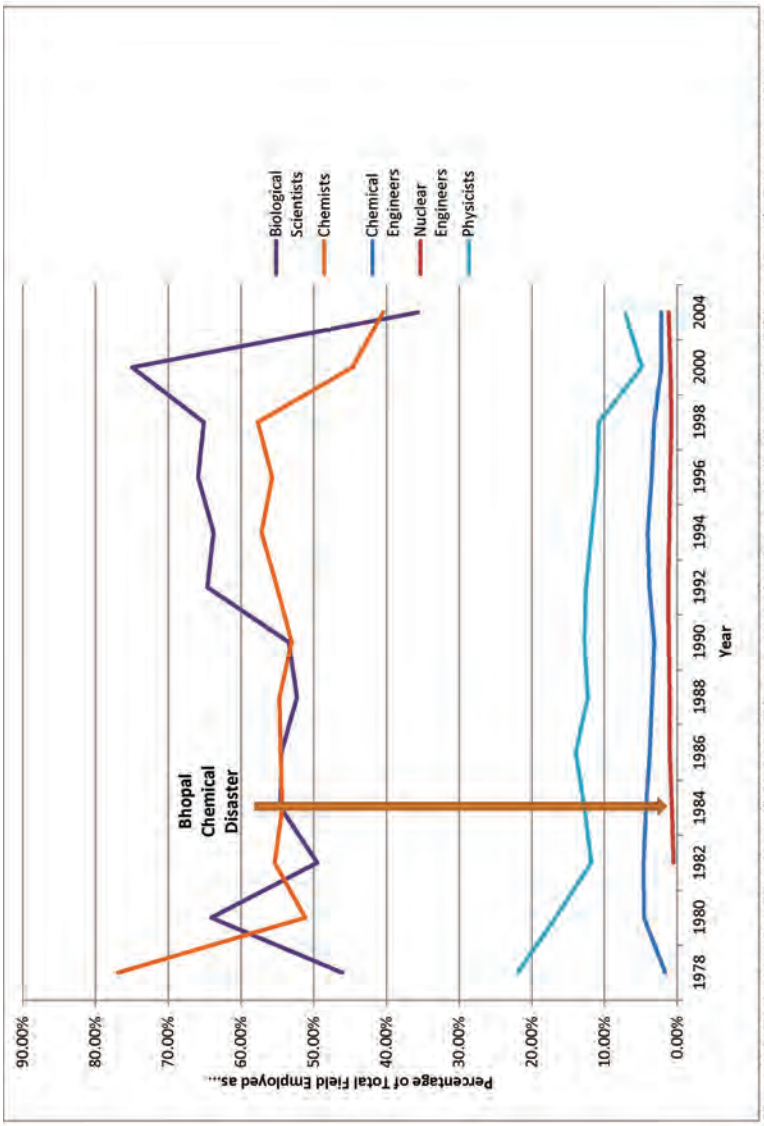

Figure 11.5. Percent employed in relevant fields in the US as a percent of engineers and scientists employed. Timing of applicable catastrophic events is shown.

Nuclear and chemical engineers represent a small proportion of total employment numbers, but the proportion remains steady throughout the time surveyed. We note that the nuclear accidents we examined lie outside the timeframe of the data collected. Similarly, the Tuskegee Syphilis Study falls outside the employment numbers, it is conceivable that the low number in 1978 could be attributed to the experiments; however, it also could be attributed to the shifting categories reflecting employment. The FMD outbreak at Pirbright also falls outside the years this data was collected.

11.3.7 General Opinion

One final method used to measure public trust in science were long-term and recently conducted general opinion surveys about science conducted by the General Social Survey (GSS) and the Pew Research Center. Figure 11.6 displays results from the General Social Survey which indicates slight increases or decreases in US public confidence in the scientific community attributable to no specific event. Both the GSS and Pew results demonstrate that trust in science has remained consistent over the past 40 years. Recent results from Pew indicate that general trust in science has decreased slightly from 2009[1159] to 2015[1160] but remains relatively high. These numbers show little if any effect from the Fukushima disaster on the public's opinion of science, in general. The US. Pew, additionally, asks questions about occupational fields, and scientists are seen as contributing "a lot" to society's well-being (only members of the military and teachers are ranked higher than scientists; doctors and engineers rank similarly to scientists.)

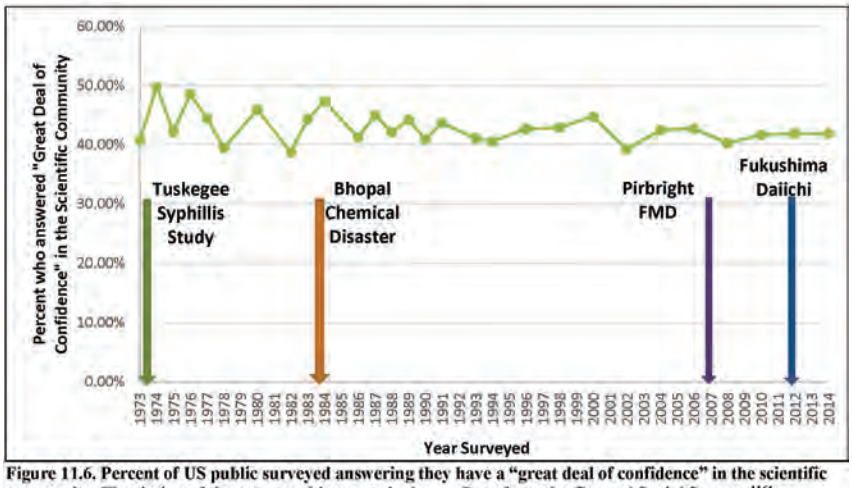

Figure 11.6. Percent of US public surveyed answering they have a "great deal of confidence" in the scientific community. The timing of the catastrophic events is shown. Data from the General Social Survey. [1161]

[1159] Pew Research Center, "Scientific Achievements Less Prominent Than a Decade Ago," July 9, 2009.
[1160] Pew Research Center, "Public and Scientists' Views on Science and Society," January 29, 2015.
[1161] General Social Survey, NORC at the University of Chicago, http://www.norc.org/Research/Projects/Pages/general-social-survey.aspx

12 Appendix I: Glossary

Absolute risk: risk given in terms of consequences per unit of real time

Adjuvants (for vaccines): substances that are added to a vaccine to boost or otherwise modify the recipient's immune response to the vaccine antigens, in order to enhance the vaccine's effects

Antibody escape mutant: a virus that has acquired mutations at antigenic sites that prevents antibody neutralization

Antigen: a substance that leads an immune system to *gen*erate *anti*bodies against it

Antigenic drift: small changes in the antigenic character of a virus that alter antibody neutralization

Antigenic shift: the phenomenon of HA or NA gene segments being exchanged between viruses in nature

Antiviral: compounds used to treat or prevent viral infections. Antivirals are a type of medical countermeasure

Assay: a general term used to describe a range of laboratory techniques that determine or measure the presence, amount, or activity of a particular substance

Atmospheric dispersion model: a model that predicts the transport of a contaminant in the air from a release site

Attenuated: a pathogen that is still able to grow in its host, but has reduced virulence. Live attenuated vaccines use an attenuated pathogen strain with extremely low virulence

Backbone (strain): an often-attenuated virus strain that contains internal gene segments of an influenza virus. A/Puerto Rico/8/1934, A/WSN/1933, and A/Ann Arbor/6/1960 are commonly used backbone strains

Biosafety: the concept of reducing the risk of natural or accidental exposure to pathogens. Biosafety measures at a laboratory reduce the probability of accidental human exposure to an agent, and where possible, reduce the consequences of such an event should it occur

Biosecurity: the concept of reducing the risk of deliberate exposure to pathogens. Biosecurity measures at a laboratory seek to guard stored pathogens against theft, diversion, or other intentional misuse, and to mitigate the consequences of such acts should they occur

Biosurveillance (surveillance): the systematic process of gathering and analyzing data that might relate to pathogen activity in the hopes of detecting disease outbreaks

Branching process model: a model of a population in which each individual in a generation produces a random number of offspring. In this report, branching process models are used to predict how many infected individuals (offspring) are produced by any infected individual in a nascent outbreak

"Bright line" boundary: an easily-applied objective rule that resolves an issue in a clear-cut manner

Biological Select Agent or Toxin: disease agents subject to federal oversight and regulation through the Federal Select Agent Program. HHS pathogens and toxins are those deemed to pose a high risk to human health, while the USDA pathogens and toxins are deemed to pose a high risk to plant or animal health. Certain "overlap" pathogens are on both lists

Biosafety Level: a laboratory ranking system, as defined in the BMBL, that assigns a level to sets of facility standards, laboratory practices, and types of safety equipment in terms of the overall containment capacity provided. Biosafety levels range from Biosafety level 1 (lowest level of containment) to Biosafety level 4 (highest level of containment)

Case Fatality Rate: the rate of deaths within the population of people infected with a pathogen

Cell culture: growing and maintaining cells isolated from an organism under controlled laboratory conditions

Cell line: a population of cells descended from a single cell, generated and maintained through cell culture

Chimeric microorganism: a microorganism created by joining nucleic acid fragments from two or more microorganisms

Codon: a triplet of adjacent DNA or RNA nucleotides that together define a specific amino acid or a stop signal during protein synthesis

Cognate antibody: an antibody corresponding to an antigenic site on the influenza virus, often used to map the different viral epitopes or to generate adaptive immune response escape mutants

Convalescent sera: the sera isolated from an animal or human after infection that contains antibodies specific to the infecting pathogen

Coronaviruses: common viruses belonging to two subfamilies (*Coronavirinae* and *Torovirinae*) of the virus family *Coronaviridae*. In this report, the term is used to describe the SARS- and MERS-CoVs not the coronaviruses that may cause the common cold.

CRISPR-Cas9: a recently developed laboratory technique used to modify genomic DNA

Crosswalking: mapping correspondences between two or more sets of data, for instance the mapping of information to knowledge gaps

Dendrograms: tree diagrams often used to organize and display association between genes or samples by grouping them into clusters

Dose-response models: models that predict the effect on an organism caused by increasing doses of a stressor. In this report, dose-response models are used to predict the probability of infection caused by a dose of a pathogen

Dose-sparing: an approach that seeks to reduce the amount of antigen required for effective vaccination

Downstream: events that occur further along in a process chain

Dual use research: a generic term that refers to civilian research that could be diverted to serve a military purpose

Dual Use Research of Concern: the official term used by the US government in documents on ensuring institutional oversight of dual-use research in the life sciences (i.e., to ensure oversight of "life science research that, based on current understanding) can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security."[1162]

Epitope: the part of an antigen to which an antibody binds

Fault tree analysis: an analytical technique in which pathways within a system that can lead to a predictable failure are described using Boolean logic

FDA-qualified animal model: an animal that has been approved for laboratory use through the FDA's Animal Model Qualification Process to accurately represent human disease processes

Fitness: the ability for an organism to survive and reproduce

Fomite: a surface or object contaminated with pathogens that can therefore serve as a vehicle in disease transmission

Forward genetic screen: the process of modifying genetic code, often *a priori*, to elucidate gene sequences responsible for particular phenotypes

Gain of Function—Research/Experiments: laboratory experiments that are reasonably expected to generate influenza or coronaviruses with enhanced growth, pathogenicity, transmission between mammals, vaccine evasion, or resistance to medical countermeasures

Gain of Function—Pathogens: the organisms subjected to Gain of Function experiments. In this report, these organisms are influenza A viruses, and SARS and MERS coronaviruses

Gain of Function—Laboratory: a workplace where Gain of Function experiments take place

(Genetic) modifications: an alteration of genetic material. See *Mutation*

Genotype: the genetic makeup of an organism

Hemagglutinin (HA): the viral protein from influenza virus that causes red blood cells to agglutinate

Hemagglutination inhibition (HI, HAI) assay: evaluates the ability for antibodies to prevent a virus from agglutinating red blood cells

Host immune modulators: substances that alter typical immune function

Host tropism: a pathogen with improved fitness in a specific host relative to other hosts

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[1162] Sorrell EM *et al* (2009) Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus. *Proc Natl Acad Sci U S A* 106: 7565-7570

in ovo: in chicken eggs

in vitro: in cell culture or in cell-free biochemical systems

in vivo: in humans or other animals

Inactivated: refers to a pathogen that has been rendered non-infectious

Influenza: a disease caused by the influenza virus

Isolate: a pure strain of a pathogen separated from a mixed culture

K: a variable used in epidemiology, defined as the variation of infectiousness between individuals

Knockout cell lines: a cell line in which one or more proteins are not expressed due to a removal or modification of the DNA encoding that protein

Markov chain model: a model in which the next state of a subject in the model is determined exclusively by its current state (and not its prior history)

Medical countermeasures: vaccines, medications, or equipment used to improve public health outcomes in response to a disease outbreak

Middle East Respiratory Syndrome: a respiratory illness caused by a coronavirus, MERS-CoV. The first known cases of MERS were reported in Saudi Arabia in 2012

Monoclonal antibody: an antibody derived from a single B-cell line, which generates an antibody to one epitope on an antigen

Monte Carlo simulation: a simulation in which the probability of various outcomes is predicted via the analysis of multiple model runs, each of which use parameter values selected at random

Morbidity: the number of individuals exhibiting disease symptoms in a given population

Mortality: the number of deaths for a given population

Mutagenesis (mutagenizing): the process of changing one or more nucleotides of DNA

Mutation(s): a change of one or more DNA nucleotides

Myalgia: muscle pain

Neuraminidase inhibitors: chemical compounds that block the viral neuraminidase enzyme, preventing virus replication. Neuraminidase inhibitors are currently used against influenza; examples of such compounds mentioned in the report include zanamivir, oseltamivir, peramivir, and laninamivir

Novel strain: a strain of a microbe distinct from any previously characterized strain

Orthomyxoviruses: a family of RNA viruses from six genera: influenza virus A, influenza virus B, Influenza virus C, Isavirus, Thogotovirus, and Quaranjavirus.

Pandemic: an epidemic occurring worldwide or over a very large area and affecting a large number of individuals

Parametric approach (parametric analysis): a modeling approach that uses multiple different parameters, each with an accompanying finite range of potential values, to describe a range of characteristics of a subject and their effect on model outcomes. In this report, a parametric approach was used to describe a variety of pathogens with a range of phenotypes manipulated under undetermined laboratory conditions to explore their influence on risk.

Parental strain: the original virus strain used as the basis for subsequent genetic modification, creating novel strains

Passaging: the process of placing a virus strain under selective pressure in cells or animals for several iterations to introduce adaptive mutations

Pathogenicity (pathogenesis): a characteristic of a virus or organism that generates harmful biological responses in the host

Phenotypic (phenotype): the physiological or measurable result of a genotype; a trait

Polyclonal antibodies: a collection of antibodies taken from the serum of an immunized individual, each of which may bind to a distinct epitope with a variety of strengths

Probabilistic risk assessment: a systematic method to assess risks, in terms of consequence and probability, for complex systems

Prophylactically (prophylactic): medications taken to prevent infection and disease

R_0: R_0 is a variable used in epidemiology, defined as the reproductive number of a transmissible pathogen (see *Transmissibility*), or the number of infected cases one infected person will create

Random mutagenesis: a laboratory technique that randomly generates mutations in DNA

Reagent: a substance or mixture of substances used in an assay or other laboratory technique

Reassortment (reassortant) (reassorting): a laboratory technique used to exchange gene segments between two or more viruses to generate new viruses. A 6:2 reassortment strain has six gene segments came from one parental strain, and two gene segments came from the other parental strain to form the new virus

Recombinant: recombining of genetic material, often from different sources, to generate new genetic sequences

Relative risk: risk of a novel event compared to the risk of a baseline event. In this report, relative risk of research on GoF pathogens is compared to risk of research on wild type pathogens (as the baseline). Relative risk is provided when the frequency of a negative event is unpredictable.

Reservoir: any organism that typically harbors a pathogen. The pathogen depends on and grows in the reservoir, and can subsequently infect other organisms in contact with the reservoir

Reverse Genetics: in this report, an approach that generates a virus from isolated genetic material

Risk: this report uses the actuarial definition of risk, the product of consequences arising from a negative event and the probability of the negative event

SEIR model: an epidemiological model that tracks the flows of hosts from the susceptible state (S) to the exposed state (E) to the infected (I) and resistant (R) states.

Serotype: viral strains described by the category of antigens displayed on the outside of the virus. Three serotypes for influenza exist, including influenza A, B, and C

Severe Acute Respiratory Syndrome: a respiratory disease caused by the coronavirus SARS-CoV. The first cases of the disease were reported in Asia in February 2003

Sialic acid moieties: in this report, residues expressed on the outside of cells which the viral HA recognizes and binds to, allowing for infection

Site-directed mutagenesis: a laboratory technique that introduces specific mutations into DNA

Stochastic: characterized by a random probability that is statistically analyzable but not predictable a priori

Sublineages: a group of related viruses descending from a common ancestor strain

Subtype: viral strains described by the set of HA and NA proteins expressed on the virus surface

Tier 1 BSAT: A select agent deemed to pose the greatest threat, which are subject to additional regulatory safety and security requirements

Titer: a measure of a virus' concentration in a given sample

Translators: the individuals who apply ("translate") fundamental research results to practice (in this report, basic research results to benefits in public health or medicine)

Transmissibility: the ability of a pathogen to spread from an initial case through a population

Vaccine (protective vaccination): a type of medical countermeasure that stimulates an immune response to prevent or mitigate future infection

Vaccine platform (platform): a set of processes and methods used to generate vaccines

Virulence: the characteristic of a virus that informs the severity of morbidity and mortality

Vivarium: a part of a laboratory complex dedicated to housing research animals

Wild type virus (strains): an unmodified organism

Yield: the remaining amount of a substance after one or more processes

Zoonotic (disease): a disease that can infect lower animals and humans

13 Appendix II. Acronyms Used

ABSL	animal biosafety level
ALF	Animal Liberation Front
alt GoF	alternatives to Gain of Function
APHIS	Animal and Plant Health Investigation Service
BARDA	US Biomedical Advanced Research and Development Authority
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BPM	branching process model
BSAT	biological select agents and toxins
BSL	biosafety level
BW	biological warfare
BWC	Biological Weapons Convention
CBRN	Chemical Biological Radiological Nuclear
CDC	US Centers for Disease Control and Prevention
CFR	case fatality rate
CFR	Code of Federal Regulations
CoV	coronavirus
CVV	candidate vaccine virus
DALY	disability-adjusted life years
DEFRA	UK Department for Environment, Food, and Rural Affairs
DOD	US Department of Defense
DURC	dual use research of concern
EAR	Export Administration Requirements/ Regulations
ELF	Earth Liberation Front
EPI	Emerging Infections Program
FBI	US Federal Bureau of Investigation
FDA	US Food and Drug Administration
FDI	Foreign Direct Investment
FEMA	US Federal Emergency Management Agency
FMD	foot and mouth disease
FSAP	Federal Select Agent Program
FTA	fault tree analysis
GAO	US Government Accountability Office
GDP	gross domestic product
GoF	Gain of Function
HA	hemagglutinin
HEPA	High-efficiency particulate air (filter)
HHS	US Department of Health and Human Services
HPAC	Hazard Prediction and Analysis Capability
HPAI	highly pathogenic avian influenza
HVAC	heating, ventilation, and air conditioning

IAH	Institute of Animal Health (UK)
IBC	Institutional Biosafety Committee
ID	infectious dose
IIM	interactive influenza model
IND	Investigational New Drug
ISIL	Islamic State of Iraq and the Levant
ITAR	International Traffic in Arms Regulations
LAI	lab acquired infection
LAV	live attenuated viruses
LD	lethal dose
LoF	Loss of Function
LPAI	low pathogenic avian influenza
MCM	medical countermeasures
MERS	Middle East Respiratory Syndrome
NA	Neuraminidase
NAIIC	Fukushima Nuclear Accident Independent Investigation Commission
NBAF	National Bio- and Agro-Defense Facility
NEIDL	National Emerging Infectious Diseases Laboratory
NIAID	US National Institute of Allergy and Infectious Diseases
NIH	US National Institutes of Health
NRC	National Research Council
NSABB	US National Science Advisory Board for Biosecurity
NSDD	National Security Decision Directive
OSHA	US Occupational Safety and Health Administration
P&I	pneumonia and influenza
pfu	plaque forming unit
PI	principal investigator
PPE	personal protective equipment
PPP	pathogens with pandemic potential
PRA	probabilistic risk assessment
RA	risk assessment
RAC	Recombinant DNA Advisory Committee
RBA	risk benefit analysis
SAR	select agent regulations
SARS	Severe Acute Respiratory Syndrome
SARS-AM	SARS animal model
SEIR	susceptible, exposed, infectious, recovered
SME	subject matter expert
SRA	security risk assessments
START	National Consortium for the Study of Terrorism and Responses to Terrorism
TEPCO	Tokyo Electric Power Company
USDA	US Department of Agriculture
USG	US Government

VSVG	vesicular stomatitis virus glycoprotein
WHO	World Health Organization
WMD	weapons of mass destruction
WoS	Web of Science database

14 Appendix III. Additional Data on the Methods of the Quantitative Risk Assessment

14.1 Additional Methodological Information Supporting the Estimate of Loss of Containment Pathways

14.1.1 Elimination of Implausible Incidents Leading to Loss of Containment

Once the list of incidents to investigate was finalized, pathways by which these incidents would lead to a loss of containment were researched. In so doing, no plausible way was found for some incidents to lead to a loss of containment and so these incidents were eliminated from quantitative modeling. These implausible incidents are listed here.

14.1.1.1 Loss of Power Should Not Occur in a Containment Laboratory

Requirements for high-containment laboratories stipulate that power must be supplied by two completely independent conduits from two sources, suggesting that two, simultaneous power outages must occur. Moreover, backup generator power is required. For this reason, a power outage would have to occur via three, extremely unlikely events. Even if a power outage is experienced, louvers in place are designed to fail safe and isolate the laboratory from the outside. Standard protocols require workers to immediately cease and secure work (e.g., by closing the sashes on any active BSCs). This event requires four completely independent, rare events to happen and therefore would be vanishingly unlikely. Also, laboratory work would not continue in a power outage suggesting that there is very little opportunity for an accident to occur during this period. Continuous sources of aerosols (animals in containment) are very dilute and pose a minimal risk even if the power and the louvers fail (see animal aerosol risk, below).

14.1.1.2 Floods Should Not Lead to a Loss of Containment

Some of the laboratories identified in our study are in areas of some flood risk (protected by levees or not). Floods are not unanticipated events, and days of warning precede a flood caused by a tidal surge from a hurricane or a river flood from excessive rain (none of the laboratories identified were in a gulley susceptible to flash floods). As learned in the interviews, in anticipation of previous hurricanes (such as hurricane Sandy) or other flooding events, the researchers sacrificed all infected animals, decontaminated the laboratory and shut it down. Even if these measures were not taken, the risk of a loss of containment from floods would be minimal. Firstly, the containment facilities of these laboratories are on the upper floors of the building so would not actually be inundated by the flood. Power, which, by requirement, must be supplied by two independent conduits and sources, would likely not be interrupted. For these reasons, even in the rare instance of a significant flood striking a containment laboratory, practices and laboratory configurations would eliminate the risk of a loss of containment.

14.1.1.3 Shipping Accidents Should Not Lead to a Loss of Containment with GoF Pathogens

In our interviews with GoF laboratories, we found that samples of GoF pathogens are not shipped out of the laboratory. Reverse genetics techniques are so routine in these laboratories that strains are "shared" between labs by the sharing of high-fidelity sequence information, the synthesis of the viral genomes and the rescue of the active viruses. Shipping is routinely used, however, in laboratories that analyze wild type samples for these samples.

14.1.1.4 Improper Inactivation Should Not Lead to a Loss of Containment Event with GoF Pathogens

Because pathogens are not shipped from GoF laboratories, the only materials that are inactivated that are taken out of the laboratory (not in the waste streams) are samples for analysis by molecular methods or microscopy. These samples are decontaminated and fixed, and the samples are placed in boxes and dunked into a decontaminant bath. There is no physical contact with the sample and the sample does not

leave the laboratory except through the waste stream. In addition, the inactivation procedures used here typically destroy the virus entirely by, for example, formalin treatment for cell fixing or Trizol treatment for RNA extraction. These procedures stand in contrast to inactivation procedures for other pathogens that inactivate them but leave them intact, such as the radiation inactivation protocol for *Bacillus anthracis*. If, however, some infectious material somehow ends up on a researchers glove during the procedure (or in the waste stream) these events are captured in the splash or waste stream incidents.

14.1.2 Elimination of Some Incident Pathways from Fault Tree Modeling

After investigating some incidents for quantitative modeling, the events were found to be so infrequent or so inconsequential (or both) that there was no need to include them in Fault Tree modeling because it was predictable that these events would not contribute to the risk of a loss of containment accident. The process for excluding those events is described here.

14.1.2.1 Liquid Waste Disposal

In this scenario, untreated liquid waste containing infectious material is dumped directly into a drain connected to a municipal sewer system. From interviews with coronavirus and influenza researchers, the primary source of liquid waste are the vacuum traps connected to aspirators in biosafety cabinets that are used to remove wash buffer and cell culture media from plates containing cells. Small volumes of liquids, from flasks and tubes, are typically autoclaved as a mode of decontamination and do not apply to this scenario. Interviews also revealed that a significant fraction of the liquid in these vacuum traps is likely to be PBS or other non-infectious buffers used to wash cells, and thus any infectious material that is aspirated is likely to be diluted several fold. As a conservative assumption, we assume the liquid to contain virus at a concentration of 1E5/mL, and presume a typical flask size of 2L that contains 1L of liquid when dumped, for a total of 1E8 virus units.

Because no sources were located that identified any human influenza or coronavirus infections from wastewater (even during influenza season when a lot more infectious material than the amount considered here enters the sewage system), in this scenario, we consider only the infection of waterfowl exposed to the wastewater, and therefore limit consideration to avian-adapted avian strains of influenza.

Immediately after dumping, residual chlorine in the municipal water system may neutralize some of the virus, which has been demonstrated with highly purified samples of HPAI H5N1 and other viruses.[1163,1164] With HPAI in idealized conditions there is about a three order of magnitude reduction of live virus, so we assume a two (log_{10}) reduction. Based on discussions with managers of large and small wastewater facilities, we conservatively estimate that samples do not separate or mix while in transit until after arriving at the wastewater treatment facility, at which point they enter a one million gallon (3.8 million liter) primary clarification tank, at which time we assume the sample fully mixes. Taken together with an initial sample volume of 1L, a conservative estimate of live virus concentration after chlorine reduction is a dilution of 1E6 virus units for a final concentration of 2.6E-4/mL.

Further processing steps may reduce the concentration more, but birds have been observed swimming in open tanks from this point on in the wastewater treatment process.[1165] Systems like anaerobic digestion and UV sterilization are effective at inactivating Avian H5N2 virus[1166], but these systems are neither universal nor used year-round so we do not consider them here.

[1163] Rice et al., 2007 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2851495/.
[1164] Cromeans et al., 2010 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2820971/.
[1165] https://youtu.be/tU416enJAes
[1166] Lucio-Forster et al. http://online.liebertpub.com/doi/abs/10.1089/ees.2006.23.897?journalCode=ees

If the city uses a combined sewer system, it may overflow during a rainstorm. Fong et al. found no significant difference in human adenovirus concentration of raw sewage and CSO overflow in Michigan,[1167] which is consistent with other studies of non-pathogenic contaminants yielding overlapping values.[1168,1169] Hence, if the live virus were poured down the drain during heavy rain, the initial sample would still roughly be diluted into 1M gallons after the initial two (log_{10}) residual chlorine reduction.

Presuming an extremely infectious avian influenza strain with an ingested ID50 of one virus unit, and a conservative assumption that a duck drinks 10mL of water while on the tank, the duck would be dosed with 2.6E-3 virus units and would be infected approximately with a probability of 2.6E-3 per incident. However, the frequency that this event occurs is also low. In order for untreated liquid waste to be dumped, two errors have to occur: the worker who last emptied the flask would have to not put disinfectant into the flask when returning it to the BSC, and the worker disposing it would have to not put additional disinfectant in it prior to dumping it. If both of these errors are rules errors with a median probability of 5E-3, and the flask is conservatively estimated to be emptied once per week, or 50 times per year, then the overall median frequency of incidents is (5E-3)*(5E-3)*(50) or 1.25E-3/year per lab. Given that event frequency and the previously calculated probability, the expected frequency of liquid-waste caused avian influenza infections is (2.6E-3)*(1.25E-3) or 3.25E-6/year per lab (three times per million years). Despite this estimation making a number of conservative assumptions, this scenario still occurs at a frequency several orders of magnitude lower than other significant contributors to risk of avian influenza, and the scenario was not included in the Fault Tree Analysis.

14.1.2.2 Pipe Leak/Burst

In this scenario, untreated liquid waste containing infectious material is dumped directly into a drain connected to a pipe that is either leaking or has burst, creating a spill out of containment inside the laboratory building. Leaks within the municipal sewer system, outside the building where the laboratory is located, are not considered here due to the differences in the dilution, ground filtration, and clean-up procedures compared to fixing an interior plumbing leak. Municipal sewer leaks would be considered under the liquid waste disposal scenario, but as mentioned in that section, no human infections of influenza or coronaviruses caused by wastewater have been reported.

In order for an exposure due to a leaking pipe to occur, two rare events must coincide: the pipe must be leaking and a laboratory worker must dump infectious liquid waste down the drain. Conservatively using the maximum pipe failure rate across all pipe sizes and failure types given in a report by the Health and Safety Executive of the United Kingdom,[1170] 1E-5 per meter per year, and a conservative expected maximum of 50m of pipe between the laboratory and municipal sewer system, the maximum expected failure rate is 5E-4/year per lab. As in the liquid waste disposal scenario, the double errors required for infectious liquid waste to enter the drain limits the median expected rate of incidents to 1.25E-3/year per lab. If the pipe leak is conservatively presumed to persist for one-fiftieth of one year prior to being fixed (approximately seven days), then the overall rate at which liquid waste dumping and pipe leaking incidents coincide is (1.25E-3)* (5E-4)*(1/50) or 1.25E-8 per year. As spills within the lab occur multiple orders of magnitude more frequently and are likely involve more concentrated virus, the pipe leak scenario was neither a significant contributor to risk nor considered further.

[1167] Fong et al. 2010. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2813034/

[1168] Metcalf and Eddy, *Wastewater Engineering, treatment and reuse*, 4th ed.

[1169] Sztruhár et al 2002, http://www.sciencedirect.com/science/article/pii/S1462075802000080

[1170] HSE UK. Failure Rate and Event Data for use within Risk Assessments. http://www.hse.gov.uk/landuseplanning/failure-rates.pdf. Last Update June 28th 2012. Accessed November 23rd 2015.

14.1.3 The Monte Carlo Framework

Monte Carlo simulations were performed for each event tree to obtain probability estimates for each potential outcome, the estimated frequency of each outcome, estimated reduction factors, and the amount of material released associated with each outcome.

As discussed in more detail in Section 6.2.5, each event tree consists of a series of potentially conditional nodes, each of which represents a step in the process at which an error or failure could potentially occur. Each node can result in either a "success" or "failure" (yes/no) outcome, and each success/failure outcome potentially affects which subsequent nodes are relevant and, in some cases, the probabilities associated with the success/failure likelihoods for subsequent nodes. In addition, for some nodes, either a success or a failure could result in a reduction in the amount of potentially infectious material available for release. In order to reflect both the statistical or probabilistic uncertainty as well as the uncertainty associated with the true value of a parameter (due to epistemic or aleatoric uncertainty), probability distributions were assigned for key input parameters for each event tree (e.g., number of opportunities, amount of material being handled) and for each node within each tree (e.g., probability of failure, reduction factors, etc.), and Monte Carlo simulations were performed.

For each fault tree, a probability distribution was assigned for the following factors:

- Number of opportunities per year for the event to occur and
- Amount of potentially infectious material available for release during any given opportunity (i.e., how much material at what concentration is being handled at any given point in time). In most cases, the amount available for release was defined as the product of two independent random variables: 1) the volume of material being handled and 2) the concentration (viral titer) of the material.

For each node in each tree, either a probability distribution or a fixed value was assigned for the following factors:

- Probability of success/failure (in some cases, this probability distribution was dependent on the outcome of previous nodes and/or the volume of material being handled), and
- Reduction factor (the fraction of infectious material is removed from the material potentially released) if a success or failure is realized (in some cases, these probability distributions were dependent on the outcome of previous nodes and/or the volume of material being handled).

For a given tree, the Monte Carlo simulation was performed by generating 2.5 million random outcome realizations. For each of the 2.5 million realizations, the following steps were performed:

1. Generated a random realization of the amount of potentially infectious material being handled, called the "Material Available for Release" (MAR).
2. For each node in the tree, assigned a probability of failure (p_{fi}, where i is the i^{th} node), either as a random realization from the distribution of possible probabilities for that node, or a fixed value if no distribution was defined. For conditional nodes (i.e., nodes with success/failure probabilities that are dependent on the result of previous nodes or the volume of material being handles), the assignment of probabilities took these results into account.

3. For each node in the tree, based on the assigned probability of failure, generated a random success or failure outcome (where the probability of success is $1 - p_fi$ and the probability of failure is p_fi).

4. For each node in the tree, based on the realized success or failure outcome, determined the reduction factor (amount by which the amount of material being handled is reduced by the success of failure of that node). The reduction factor for a given realization was generated either as a random realization from the distribution of possible reduction factors associated with a success or failure outcome for that node, or a fixed value if no distribution was assigned. Note that for some trees, different reduction factors were assigned based on the type of potential exposure (e.g., fomite or aerosol exposure).

5. Based on the series of successes and failures for a given realization, determined whether an exposure occurred and, if so, the type of exposure that occurred (e.g., personal aerosol exposure, hand fomite exposure, subcutaneous exposure, etc.). This determination was based on the description of each tree.

6. For each realization that resulted in an exposure, computed the overall reduction factor as the product of reduction factors realized for each node, as well as the mass of potentially infectious material involved in the exposure. The mass of material involved in the exposure ("Q") was computed as the MAR for a given realization multiplied by the overall reduction factor for that realization. Note that for some trees, overall reduction factors and resulting Q values were computed separately for different types of exposures (e.g., fomite or aerosol exposure).

All results from every one of the 2.5 million passes through the tree were stored, allowing various summary statistics to be computed. For each node, the observed proportion (estimated probability) of failure, the average and standard deviations for the observed (realized) reduction factors when the node was successful and the average and the standard deviations for the observed reduction factors when the node was a failure was computed. Note that reduction factor averages and standard deviations were computed for all relevant types of exposure (e.g., aerosol, fomite).

For each unique "trace" through the tree (i.e., each unique pattern of successes and failures), the observed proportion of the 2.5 million runs that resulted in that unique trace, representing the estimated probability of that trace, was computed. In order to more fully understand the uncertainty associated with the estimated probability for the trace, an additional set of calculations was performed for each trace, wherein the probabilities that had been assigned to each node for a given pass through the tree (i.e., the pfi values) were used to compute the probability of the trace (i.e., the probability of that trace's unique pattern of successes and failures) for each of the 2.5 million passes through the tree. This approach generated a distribution of 2.5 million probabilities per trace. The average and standard deviation as well as the 1st, 5th, 50th, 95th, and 99th percentiles were computed. Also, the averages and standard deviations of the overall reduction factors associated with the trace were computed. Note that reduction factor averages and standard deviations were computed for all relevant exposure types. Lastly, the averages and standard deviations of the Q values (the product of MARs and the reduction factors) associated with the trace were computed. Q value averages and standard deviations were computed for all relevant exposure types.

Because numerous traces resulted in the same "exposure outcome" (e.g., different series of successes and failures could all lead to a hand fomite exposure), an additional summary table was created that summarized the results across all traces associated with an exposure outcome. For each unique exposure outcome, the following statistics were computed and summarized:

- Observed proportion of the 2.5 million runs that resulted in the exposure outcome, which represents the estimated probability of the exposure outcome and was computed as the sum of the probabilities (proportion of observed occurrences) for all unique traces that resulted in the exposure outcome. For example, if three different traces all resulted in an environmental aerosol exposure, the probability of an environmental aerosol exposure was computed as the sum of the probabilities for each of those three traces.
    - Similarly, the uncertainty in the estimated probability for a given exposure outcome was captured by computing the probability of that outcome for each of the 2.5 million passes through the tree. For a given pass, the probability of the exposure outcome was computed as the sum of the probabilities for each trace that resulted in the given exposure outcome, which generated a distribution of 2.5 million probabilities for the exposure outcome. The average and standard deviation as well as the 1st, 5th, 50th, 95th, and 99th percentiles were then computed for this probability distribution.

- The averages and standard deviations of the overall reduction factors associated with the exposure outcome (for all relevant exposure types).

- The averages and standard deviations of the Q values associated with the exposure outcome (for all relevant exposure types).

- The range of potential frequencies of occurrence for each exposure outcome was also computed. The first step was to generate 2.5 million realizations of the number of opportunities, based on the probability distribution for the number of opportunities per year for the event tree. The product between the ith opportunity count and the ith probability of the exposure outcome was then calculated for each of the 2.5 million opportunity counts and probabilities, to generate 2.5 million "expected frequencies". The average and standard deviation as well as the 1st, 5th, 50th, 95th, and 99th percentiles of these frequencies were then computed.

14.1.4 Human Reliability Assessment in Biological Laboratories

Many years of continuous improvement in containment laboratory design has reduced the failure of the many mechanical containment features to rates below that of human reliability. A significant fraction of this reduction in failure rate comes from the mechanical redundancies, interlocks, and alarm systems that require a cascading series of improbable events to occur prior to a loss of containment event. In addition, the interlocks and alarms provide a visual, auditory, or physical alert that a failure has occurred, converting previously covert failures into overt ones, and allowing workers present in the facility to cease work and rectify the failure or error condition prior to a loss of containment event.

In contrast to these mechanical failures, human errors often remain covert, and a single human error can inadvertently subvert many mechanical or physical safety features simultaneously. For example, while a typical pass-through autoclave used in a BSL-3 facility may contain a temperature readout, pre-programmed cycles to ensure proper time, alarms that report failure conditions, and a physical interlock that prevents the clean side doors from opening unless a complete, successful cycle has finished, an operator that does nothing more than overload the autoclave due to naiveté or momentary forgetfulness can result in still-contaminated material leaving the containment suite. In addition to human errors subverting safety features, due to the design of the mechanical safety features, many loss-of-containment scenarios are unlikely to occur unless precipitated via human error.

Finally, human errors can exacerbate a mechanical failure or loss of containment event. For example, workers who misinterpret, ignore, or otherwise silence alarms, whether caused by misbehavior or

ignorance, convert a routine response to a mechanical failure into a potential covert loss of containment event. Workers who experience potential exposures and ignore the established response protocol due to a self-assessed belief that the risk of the exposure is low, or, conversely, a fear of shame and consequences should the incident be reported, can increase the chance that an exposure leads a laboratory-acquired infection. Moreover, should a worker fail to follow an isolation protocol after an exposure, a laboratory-acquired infection may initiate a local outbreak.

For these reasons, assessing human reliability in containment labs is a critical component to modeling the risk of loss-of-containment events. Although some human error rates are available for specific types of biological laboratory accidents, no comprehensive Human Reliability Analysis (HRA) study has yet been completed for a biological laboratory. Assigning approximate human error probabilities for specific event nodes in the model accident trees required finding suitable proxies for accidents in other fields.

Operations research conducted to facilitate the assignment of data from one context for human errors to a another has shown that, as a first approximation, human errors can be grouped into a few generic error categories by behavior type, with associated "rule of thumb" accident error ranges.[1171,1172,1173] These values are then typically refined by researchers, for example based on employee error rates on equipment simulators used during training or surveyed rates during operation.[1174]

The classification system used here is derived from nuclear power plant HRA studies, and consists of three categories: rule-based, skill-based, and knowledge-based errors.[1175,1176] Table 14.1 below summarizes the classification system adapted for use in this study.

[1171] In addition to unstructured searches for operations research literature, a systematic search for all sources mentioned in the bibliography of a recent textbook on HRA studies was conducted: Anthony J. Spurgin, *Human Reliability Assessment: Theory and Practice* (Taylor & Francis Group, 2009).

[1172] See for instance: Charles P. Shelton, "Human Interface/Human Error," 18-849b Dependable Embedded Systems, Spring 1999, http://users.ece.cmu.edu/~koopman/des_s99/human/. Accessed August 3, 2015. Based on data from Barry Kirwan, *A Guide to Practical Human Reliability Assessment* (London: Taylor and Francis Ltd., 1994).

[1173] Other factors, such as the amount of time available to rectify a mistake before an accident occurs, can then be incorporated as adjustment factors. See for example: Ronald L. Boring, David I. Gertman, "Human Error and Available Time in SPAR-H," CHI 2004 Workshop on Temporal Aspects of Work for HCI," p. 3, http://www.aeso.ca/downloads/2009-02-06_Study_of_Human_Error_rates.pdf. Accessed August 3, 2015.

[1174] Pierre Le Bot, "Human reliability data, human error and accident models – illustration through the Three Mile Island accident analysis," *Reliability Engineering and System Safety* 83 (2004): p. 154.

[1175] Electric Power Research Institute (EPRI), "Systematic Human Action Reliability Procedure (SHARP)," EPRI NP-3583, Project 2170-3, Interim Report, June 1984, A-8, http://www.epri.com/abstracts/Pages/ProductAbstract.aspx?ProductId=NP-3583. Accessed August 3, 2015.

[1176] The four-tier categorization process used in the following source was also consulted to define cases: Scott Shappell, Doug Wiegmann, HFACS Analysis of Military and Civilian Aviation Accidents: A North American Comparison, ISASI 2004 http://www.asasi.org/papers/2004/Shappell%20et%20al_HFACS_ISASI04.pdf.

Table 14.1. General Human Error Types as Applied to a Biological Laboratory

Human error type[1177]	Definition	Error rate improves with	Accident probability range	Examples
Rule-based	Errors in following instructions or set procedures, accidentally or purposefully	Redundant checking; Written rules vs. oral instructions[1178]	5E-4 to 5E-2, log uniformly distributed	Omitting a required PPE item, violating isolation
Skill-based	Errors involving motor skills involving little thought	Redundant processes; Practice	5E-5 to 5E-3, log uniformly distributed	Cutting oneself with a sharp object, creating a splash while pipetting
Knowledge-based	Errors stemming from a lack of knowledge or a wrong judgement call made based on a lack of experience	Experience	5E-3 to 5E-1, log uniformly distributed	Identifying an incorrectly labeled package as actually hazardous, choosing the proper centrifuge tube

A search for human error data in other fields was conducted to verify the validity of the chosen error ranges and to refine range estimates in specific cases. Data sets and associated reports on human errors in

[1177] Electric Power Research Institute (EPRI), "Systematic Human Action Reliability Procedure (SHARP)," EPRI NP-3583, Project 2170-3, Interim Report, June 1984, A-8. http://www.epri.com/abstracts/Pages/ProductAbstract.aspx?ProductId=NP-3583. Accessed August 3, 2015.

[1178] Based on numbers and discussion in: A. D. Swain, H. E. Guttmann, "Handbook of Human Reliability Analysis with Emphasis on Nuclear Power Plant Applications: Final Report," NUREG/CR-1278, SAND80-0200, August 1983, http://pbadupws.nrc.gov/docs/ML0712/ML071210299.pdf. Accessed August 3, 2015.

the nuclear,[1179,1180,1181] aerospace,[1182] aviation,[1183,1184,1185,1186] medical,[1187,1188,1189,1190] and hazardous materials sectors,[1191] in the workplace,[1192,1193] and with motor vehicles,[1194] were compiled and reviewed.

Extracting useable human error rates from the available accident data requires knowing the total number of accidents caused by human errors (the numerator) and the total number of operations that could have led to an accident (the denominator). In general, human error data suffers from a lack of values for the denominator. Accidents per year are often tallied in the literature, and the number of said accidents attributable to human error are sometimes available, but very few studies can provide a count of the total

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[1179] A. D. Swain, H. E. Guttmann, "Handbook of Human Reliability Analysis with Emphasis on Nuclear Power Plant Applications: Final Report," NUREG/CR-1278, SAND80-0200, August 1983, p. 15-14, p. 6-17, p. 20-38, p. 15-5, http://pbadupws.nrc.gov/docs/ML0712/ML071210299.pdf. Accessed August 3, 2015.

[1180] Electric Power Research Institute (EPRI), "Systematic Human Action Reliability Procedure (SHARP)," EPRI NP-3583, Project 2170-3, Interim Report, June 1984, A-8, http://www.epri.com/abstracts/Pages/ProductAbstract.aspx?ProductId=NP-3583. Accessed August 3, 2015.

[1181] M. K. Comer, D. A. Seaver, W. G. Stillwell, C. D. Gaddy, "Generating Human Reliability Estimates Using Expert Judgement Volume 2. Appendices," NUREG/CR-3688/2 of 2, SAND84-7115, November 1984, p. C-1 – C-10, http://prod.sandia.gov/techlib/access-control.cgi/1984/847115-2.pdf. Accessed August 3, 2015.

[1182] Chandler F, et. al. (2010) "NASA Human Error Analysis," http://www.hq.nasa.gov/office/codeq/rm/docs/hra.pdf. Accessed August 3, 2015.

[1183] Garibay A, Young J (2013) "Reducing General Aviation Accidents By Utilizing Airline Operational Strategies," Aviation Technology Graduate Student Publications, Paper 25: 6, http://docs.lib.purdue.edu/cgi/viewcontent.cgi?article=1019&context=atgrads. Accessed July 1, 2015.

[1184] Shappell S (2006) "Human Error and Commercial Aviation Accidents: A Comprehensive, Fine-Grained Analysis Using HFACS," http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA463865. Accessed July 1, 2015.

[1185] Maurino D (2000) "Human Factors and Safety Management: The Role of the Regulator," Flight Safety and Human Factors – ICAO, 14th Annual FAA/CAA/TC Human Factors in Aviation Maintenance Symposium, Vancouver, Canada. http://www.faa.gov/about/initiatives/maintenance_hf/library/documents/media/mx_faa_%28formerly_hfskyway%29/14th_symposium/human_factors_and_safety_management_the_role_of_the_regulator.pdf. Accessed July 1, 2015.

[1186] Shappell S, Wiegmann D (2004) "HFACS Analysis of Military and Civilian Aviation Accidents: A North American Comparison," Australian Society of Air Safety Investigators ISASI, http://www.asasi.org/papers/2004/Shappell%20et%20al_HFACS_ISASI04.pdf. Accessed July 1, 2015.

[1187] Committee on Quality of Health Care in America, Institute of Medicine, *To Err is Human*, eds. Linda T. Kohn, Janet M. Corrigan, Molla S. Donaldson (Washington: National Academies Press), p.1, 28, 31-34.

[1188] Marx D (2001) "Patient Safety and the "Just Culture": A Primer for Health Care Executives," http://www.safer.healthcare.ucla.edu/safer/archive/ahrq/FinalPrimerDoc.pdf. Accessed July 1, 2015.

[1189] Centers for Disease Control and Prevention, "Inpatient Surgery," April 29, 2015, http://www.cdc.gov/nchs/fastats/inpatient-surgery.htm. Accessed July 1, 2015.

[1190] Benger JR, Lyburn ID (2003) "What is the effect of reporting all emergency department radiographs?," Emergency Medicine Journal: 40–43, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1726029/pdf/v020p00040.pdf. Accessed August 3, 2015.

[1191] U.S. Department of Transportation, Pipeline and Hazardous Materials Safety Administration, "Top Consequence 2005-2009: Hazardous Materials by Commodities & Failure Modes," Issue 3, September 1, 2011, p. 9, http://www.phmsa.dot.gov/pv_obj_cache/pv_obj_id_3340E5EE847704EA2F3FBF73F59757A32478070O/filename/Top%20Consequence%20Hazardous%20Materials%20Commodities%20Report.pdf. Accessed July 1, 2015.

[1192] United States Department of Labor, Bureau of Labor Statistics, "Occupational Injuries/Illnesses and Fatal Injuries Profiles," data retrieved at http://data.bls.gov/gqt/InitialPage on July 16, 2015. The datasets are split between non-fatal and fatal injuries. For non-fatal injuries, select either "Case and Demographic Numbers" or "Case and Demographic Incidence Rates". Then under "Characteristic type", select either: "Source of injury/illness" to get the equipment or harmful substance leading to the accident (ex. acids), or "Event or exposure" to get the type of incident that occurred (ex. bitten and struck by animal). The event types studied were: "Bitten and struck by animal," "exposure intact skin, eyes, or other exposed tissues," "exposure scratch or other open wound," "exposure through medical injection," "exposure unintentional needlestick, sharp injury," and "needlestick without harmful substance." The work sectors considered were "all," "health care and technical," and "computer, engineering, and science." For fatal injuries, the datasets extracted were fatal occupational injuries for biological scientists (code 19102x).

[1193] Brown A, Patterson D (2001) "To Err is Human," Proceedings of the First Workshop on Evaluating and Architecting System Dependability http://roc.cs.berkeley.edu/papers/easy01.pdf. Accessed August 3, 2015.

[1194] National Highway Traffic Safety Administration (NHTSA), "An Examination of Driver Distraction as Recorded in NHTSA Databases," Traffic Safety Facts: Research Note, p.1, http://www-nrd.nhtsa.dot.gov/Pubs/811216.pdf. Accessed July 1, 2015.

number of procedures per year that could have led to accidents, so a rate is impossible to obtain. Gathering denominator data is difficult and expensive, often requiring direct observation. For instance, biological research workplace accident data by accident type retrieved from the Bureau of Labor Statistics could not be used, in part because it was impossible to determine how many actions were undertaken per year that could have led to injuries such as "falls, slips, [or] trips."[1195] For several sectors–such as workplace accidents, motor vehicle accidents, and hazardous shipment accidents– HRA studies are rarely conducted because insurers and regulators are primarily interested in determining risk group and risk factors based on number and severity of accidents per year.

Sources with a large sample of total accidents, such as the aforementioned Bureau of Labor Statistics dataset, also often lacked the level of granularity in accident types needed to ensure accident situations were analogous to plausible incidents in high-containment laboratories. Even when considering the list of accidents relevant to biology, this comparison would be dubious. For example, a marine biologist slipping and injuring themselves while working along the shore would have been categorized as a "biologist – falls, slips, trips" event, but this situation bears little resemblance to the analogously categorized biologist slipping and injuring themselves while working in a high-containment laboratory. Since the dataset categories compressed a wide range of accident types and accident variants together, it was not possible to combine the data from this large dataset with human error studies with small sample sizes to obtain trustworthy accident rates applicable to high-containment laboratory work.

In other fields, such as surgical medicine and especially commercial and military aviation, routine operation involves an extremely large number of individual actions and non-actions, complicating the extraction of useable denominator values. To take an example from commercial aviation, roughly two errors are committed *per flight*, the vast majority of which have no consequences and are not noticed by the flight crew.[1196] In addition, accidents in these contexts are often complex situations caused by a combination of human and mechanical failures exacerbated by abnormal operating conditions.[1197] In such cases, the number of incidents solely attributable to human error is difficult to obtain. As a result, human error rates in potential proxy fields often could not be reliably determined.

Notable exceptions were found in reports for the nuclear and aerospace industries, where the potential catastrophic consequences of errors has motivated detailed HRA research. The human error data used in this section was mostly derived from these sources, principally from the Electric Power Research Institute (EPRI) "Systematic Human Action Reliability Procedure (SHARP)" document NP-3583.[1198] These were complemented by values taken from the Sandia Laboratories NUREG/CR-1278 "Handbook of Human Reliability Analysis with Emphasis on Nuclear Power Plant Applications".[1199] More specifically, the sections of the NUREG/CR-1278 used were those on omitting steps listed out on written instructions,

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[1195] United States Department of Labor, Bureau of Labor Statistics, "Occupational Injuries/Illnesses and Fatal Injuries Profiles," data retrieved at http://data.bls.gov/gqt/InitialPage on July 16, 2015.

[1196] Maurino D (2000) "Human Factors and Safety Management: The Role of the Regulator," Flight Safety and Human Factors – ICAO, 14th Annual FAA/CAA/TC Human Factors in Aviation Maintenance Symposium, Vancouver, Canada. http://www.faa.gov/about/initiatives/maintenance_hf/library/documents/media/mx_faa_%28formerly_hfakyway%29/14th_symposium/human_factors_and_safety_management_the_role_of_the_regulator.pdf. Accessed July 1, 2015.

[1197] See for instance data in Table 2 of: Scott Shappell, "Human Error and Commercial Aviation Accidents: A Comprehensive, Fine-Grained Analysis Using HFACS," July 2006, p. 7, http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA463865 Accessed July 1, 2015.

[1198] Electric Power Research Institute (EPRI), "Systematic Human Action Reliability Procedure (SHARP)," EPRI NP-3583, Project 2170-3, Interim Report, June 1984, A-8, http://www.epri.com/abstracts/Pages/ProductAbstract.aspx?ProductId=NP-3583. Accessed August 3, 2015.

[1199] Swain AD, Guttmann AE (1983) "Handbook of Human Reliability Analysis with Emphasis on Nuclear Power Plant Applications: Final Report," NUREG/CR-1278, SAND80-0200 http://pbadupws.nrc.gov/docs/ML0712/ML071210299.pdf. Accessed August 3, 2015.

misremembering oral instructions, misreading labels, and detecting errors.[1200]

Three general types of errors were most frequently used in the analysis. The first of these was the rule error, which were errors incurred in any laboratory task where a prescribed procedure or rule applied to a task, as in, for example, wearing PPE, including safety features in a centrifuge, or washing one's hands when leaving a laboratory. For general rule errors where no specific cause of the failure could be assigned, the entire failure rate range listed in the source, 5E-4 to 5E-2 per attempt, was used, distributed log normally (i.e., uniformly distributed on the exponent). When a specific type of rules error failure with an assignable probability was believed to be the most likely cause of the error, it was used as the mode of a log triangular distribution over the range. This parameter range was used, for example, in the application of an error occurring while following a protocol of more than ten steps to the failure to properly package a shipment of infectious material. Rules errors include failures to follow rules due to any cause, including ignorance, forgetfulness, or willful disobedience.[1201]

The second general error used was the skill-based error, which are errors involving motor skills, in, for example, handling a sharp instrument during necropsy. In order for this type of error to apply, the task must be one where the motor skill of the individual would improve over time with practice. For these errors, the entire failure rate range listed in the source, 5E-5 to 5E-3 per attempt, distributed log normally, was used. Skill errors were not assigned to basic motor tasks a worker would also attempt outside of a laboratory, such as holding an object without dropping it, or walking without tripping, as these are not motor tasks for which the failure rate would likely decrease with worker practice.

The third general category of error was the knowledge error, which were errors caused by intellectual naivety or misunderstanding, and are a type of error whose probability decreases through experience with the topic. For example, PAPR failures, such a disconnected tube or low battery, would be self-announcing via a silent fan and reduced airflow. Workers with extensive experience with PAPRs would be more likely to immediately notice the change in sensation versus a worker who had just begun using PAPRs. Like the rules error, for general knowledge errors where no specific cause could be assigned, the entire failure rate range from the source, 5E-3 to 5E-1 applied and was log normally distributed. When a specific cause could be identified, that probability was assigned to the mode of a log triangular distribution over the range. In certain cases, the upper limit of the range was restricted to a lower value of 1E-1, reasoning that only the persons least experienced with the task would fail at the original limit of 5E-1, and interviews with practicing influenza and coronavirus researchers repeatedly revealed that all workers in the high containment laboratories had practice and training in lower containment before entering the laboratory.

In addition to these three general ranges, which applied to the majority of the errors appearing in the event trees, two other errors were used. The first was the previously mentioned failure to follow a protocol of greater than ten steps, which applied when an error was committed in attempting a task for which a detailed protocol is likely to be present. The second was a failure due to misreading a label, which applied, for example, when a label on a package was misunderstood and resulted in the package being mis-delivered.

It should be noted that these probabilities, as applied, are the best, but still flawed, approximation of the risk of accidents or errors as committed in a biological laboratory. Biological researchers tend to be highly skilled with many years of experience prior to entering a high containment space, which could lead

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[1200] Ibid.

[1201] In the biosafety section, intentional violations of the rules are assumed to be committed wihtout malicious intent, but instead were due to laziness or the false assumption by the worker that the risk of accident was negligible enough not to bother with the required procedure or equipment, a type of violation seen in historical accident reports and mentioned by interviewees.

to errors being committed at rates near the lower limit of the ranges used here. While the ranges used have been carefully calibrated to represent a general range of errors of this type across industries, possibly, biological researchers commit errors at frequencies outside the range given due to their level of training and education. Any primary research into the types of errors and their frequencies committed in biological laboratories has the possibility to increase both the precision and accuracy of the error rates incorporated into the fault tree analysis in this report, reducing uncertainty. Given the significance of human error in driving risk, and the possible consequence of an accident in a containment laboratory, such a study would likely have great utility.

14.1.5 Further Information on Modeling Infection Risk Caused by Fomites

14.1.5.1 Summary

A stochastic Markov model was developed to predict the likelihood of an outbreak initiating after a laboratory worker leaves containment with virus on his or her person. The model tracks the contamination through the paths it must take to result in infection of the initial laboratorian, of one or more household or community members, or of avian species on a commercial farm (or any combination of the three) (Figure 14.1). All infections are the result of internalization of the virus from a contaminated surface or body part; that is, this is a model of contamination transference and subsequent infection, not a model of contagious transmission. Any avian infections resulting from this model are assumed to spread throughout the flock and cause large outbreaks on the scale of historical avian influenza outbreaks. Epidemiological spread of human infections is modeled in the branching process model for local outbreaks and the IIM SEIR model for global outbreaks.

The transference model utilizes Monte Carlo simulations that string together the likelihoods of a number of possible actions that would lead to internalization, spread, or removal of the virus (namely, contacting your eye, nose, or mouth; physical contact with another person; contacting surfaces and fomites through regular activity; handwashing; and showering). The frequency of each of these events is described as a rate per minute, and for each minute of model time a random draw from a binomial distribution (with a probability equal to the event rate) determines if the event occurs. The viability of the virus also decreases according to its half-life on skin or nonporous surfaces over the course of the model time. Each contact event, whether to a fomite, surface, or person, transfers a certain fraction of the virus, based on data collected from published studies on transfer of viral material. Human infection occurs when viral contamination on a person's hand enters their mucosal membranes of the eye, nose, or mouth, and is dose-dependent based on the calculated amount of virus present at the time of inoculation.

For an animal infection to occur, the primary laboratorian must visit a farm housing a susceptible species, at which point it is assumed that all of the virus is inoculated into the animal. For animal contact to occur, the worker may need to violate quarantine protocols, which occurs at a specific probability, after which visits to an animal facility occur at a predetermined rate, as with the events above.

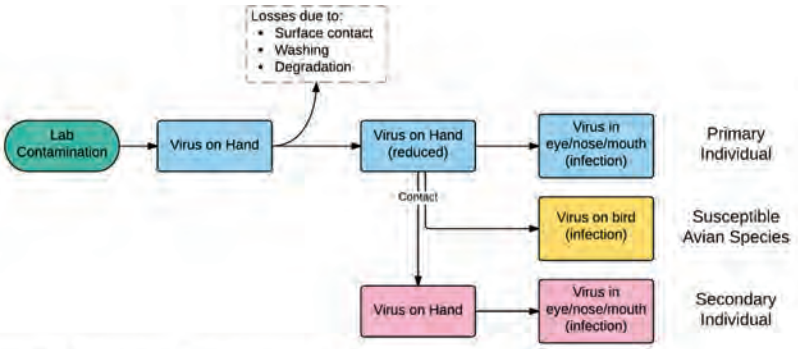

Figure 14.1. Schematic of the transference and infection model.

14.1.5.2 Model Structure

The transference model simulates three types of events: spreading of contamination from an individual's hands to another person or animal; loss of virus onto surfaces through contact, washing, or virus degradation; and inoculation of a susceptible species, either one's self (through inoculation of mucosal membranes in the eye, nose, or mouth from contamination on an individual's hands) or an avian species at a poultry farm. In every unit of model time, each of these events may or may not occur, based on a specified rate of occurrence. Through repeated such events, the model predicts whether the laboratorian causing the loss of containment is infected, how many other people in the laboratorian's household or the community are infected, and if any avian species are infected (in the case of avian influenza). These calculations are performed for a number of simulated releases, allowing determination of a frequency of each consequence occurring.

The model functions by evaluating the likelihood of each event happening during every minute from the initial release (i.e., exit of containment by a worker carrying contamination) through the next 24 hours. The likelihood of each event occurring in any given minute is determined by the average rate of occurrence, and is independent of other events happening. Whether a given event occurs within a given minute is determined by a random draw from a binomial distribution with probability p = events per minute. Minutes were chosen as the unit of model time so that all event rates would be less than one.

Model events occur sequentially, that is, two events cannot happen simultaneously, even within a given minute. The events are therefore evaluated in a predetermined order within each minute modeled, as follows:

1. Worker touches his or her eye, nose, or mouth
2. Worker touches household member
3. Worker touches community member
4. Worker touches surfaces
5. Worker washes hands
6. Worker showers
7. Remaining virus is fractionally degraded

Each of the possible events is described in further detail below.

14.1.5.2.1 Spread of Virus

Each model simulation begins with an amount of virus (in pfu) contaminating a laboratorian's hand as he or she leaves the containment facility. This viral contamination can be spread to other individuals in the worker's household or in the community at large. The spread of virus is calculated as follows. For every minute modeled, the worker may come into contact with a family member and may also contact a member of the community. Whether either of those contacts is made is based on specified frequencies of occurrence for each type of contact. A random draw from a binomial distribution determines whether the contact happens in each step of the model time.

Contact with other individuals is assumed to be through hand-to-hand contact (e.g., handshakes), as that is a common form of personal contact and the type most likely to cause subsequent inoculation through touching of one's face (it is more likely someone will touch their face with a contaminated hand than a contaminated forearm or shoulder). When contact occurs, a fraction of the virus is transferred to the recipient's hand. The initial worker can spread viral material to several other individuals through multiple contact events over the course of the model run (within each minute modeled, however, the worker can only touch at most one household member and one community member). Further spread of the virus from those contacted to additional generations of recipients is not followed by the model.

14.1.5.2.2 Loss of Virus

In addition to spreading of contamination from person to person, viral material can be lost through touching inanimate objects and surfaces, washing hands and showering, and through the natural decay that occurs in viruses on surfaces. Therefore, even without contacting other individuals, the likelihood of a contaminated laboratorian infecting himself or herself will diminish over time as these events occur.

As with the contact events, viral loss through touching surfaces, handwashing, and showering occurs based on specified rates. Each event has a separate rate, and for each minute modeled a draw from a binomial distribution determines whether the event happens. Virus loss events occur within a single unit time in the following order: contact of surfaces, handwashing, showering; all loss of virus events occur after spreading events (described above).

Degradation of the virus occurs as the final event of every unit of model time (unlike the previously described events, which may or may not occur). The amount of virus remaining is calculated as an exponential decay function based on the amount of virus at the end of the unit of model time (after all contact and washing events) and the half-life of the virus on skin, as demonstrated in the following equation:

$$N_t = N_0 2^{-t/t_{1/2}}$$

Where:

t = Duration of time passed (i.e., one minute)
$t_{1/2}$ = Half-life of virus on skin (in minutes)
N_0 = Amount of virus before degradation
N_t = Amount of virus remaining after degradation

14.1.5.2.3 Human Infection

The initial laboratorian can become infected by inoculating himself or herself in the mucous membranes of the eye, nose, or mouth. As with other contact events, in each minute modeled the worker may touch his or her face, depending on a random draw from a binomial distribution based on a specified rate of face touching. When a person touches his or her face, a fraction of the virus is transferred to the mucous membrane (the same fraction as is transferred during hand-to-hand contact). Virus accumulates in the body with each face contact event, and all face locations (eye, nose, and mouth) are considered as one. At the end of the modeling run, the total amount of virus accumulated in the body is used to determine the probability of infection, based on a probit dose-response function.

Secondary recipients of the laboratorian's contamination also become infected through self-inoculation following face touching events. However, instead of modeling each additional contaminated person completely, a single mock individual is modeled and used to determine the amount of virus remaining on a secondary person's hand at any point in time. For each secondarily contaminated individual, the time until the first face contact event is determined by a random draw from an exponential distribution with parameter λ = 1/rate of contact.[1202] The fraction of virus remaining on the mock individual at this time post-contamination is then used to calculate the amount of virus remaining on the contaminated individual, based on the amount that was transferred during the hand-to-hand contact event. A fraction of the virus on the individual's hand is internalized (the same fraction that is transferred during hand-to-hand contact) and the probability of infection is calculated based on a probit dose-response function. The calculation of the internalized dose for secondary recipients of contamination ignores subsequent face-touching events; however, in most cases the first contact event contributes the vast majority of internalized virus, and the contribution to total dose of subsequent events is negligible.

14.1.5.3 Animal Infection

The initial laboratorian can infect animals if he or she visits a farm location housing susceptible species. Only farmed poultry species are considered in the model, as contacts between people and wild birds are exceedingly rare. The model estimates whether an outbreak in birds is initiated only, not the size of the outbreak.

Only a certain portion of laboratory workers will ever contact a susceptible avian species. Workers who do contact susceptible species do so at a specified rate. For each minute modeled, whether or not animals are contacted by the worker is evaluated by a random draw from a binomial distribution. Additionally, staff working with avian influenza are required to follow a five-day quarantine period[1203] wherein they cannot contact any avian species, which is modeled with a specified rate of failure to adhere to protocol.

When a worker does contact an animal, all virus on the worker's hand at the time of contact is assumed to be inoculated into the animal, and the probability of infection of the animal is determined by a probit dose-response function. Animal contact is the last event evaluated within each minute modeled, and thus the amount of virus transferred to the animal is the amount remaining on the worker's hand after all prior contact, washing, and degradation events.

1202 For any event that happens randomly at an average rate r, the durations of periods between each event follow an exponential distribution with rate parameter $\lambda = 1/r$.

1203 Centers for Disease Control and Prevention, (2013c) Interim Risk Assessment and Biosafety Level Recommendations for Working With Influenza A(H7N9) Viruses.

14.1.6 Sources Used to Identify Incident Scenarios to Include in the Study

In this study, the following previous laboratory accident risk assessments were analyzed:

- National Bio- and Agrodefense Facility (NBAF) Updated Site-Specific Biosafety and Biosecurity Mitigation Risk Assessment (DHS 2012),
- NBAF Site-Specific Biosafety and Biosecurity Mitigation Risk Assessment (DHS 2010),
- BioSquare Phase II (NEIDL) Supplemental Final Environmental Impact Report (Boston U 2013),
- Biological Defense Research Program Final Programmatic EIS (DOD 1989),
- Final Revised Environmental Impact Study (EIS) for the Proposed Construction and Operation of a BL3 Facility at LLNL (DOE 2008),
- Environmental Assessment for the Proposed Construction and Operation of a BL3 Facility at Los Alamos National Laboratory (DOE 2002),
- Final EIS, Rocky Mountain Laboratory Integrated Research Facility (NIH 2004),
- Final EIS for George Mason University Biomedical Research Laboratory (NIH 2008),
- Final EIS for University of Louisville Center for Predictive Medicine Biodefense EID Regional Biocontainment Laboratory (RBL) (NIH 2007),
- EIS for Colorado State RBL (NIH 2003), and
- Evaluation of Health and Safety Risks of the New US Army Medical Research Institute of Infectious Disease High-Containment Facilities at Fort Detrick [NRC 2010]

Most of these studies were completely qualitative and lacked descriptions of possible loss-of-containment scenarios. These studies provided some semi-quantitative calculations based on hypothetical scenarios with notional parameters. For these qualitative studies, the scenarios that were considered were simply noted for inclusion. The NBAF studies were quantitative because pathways, frequencies and consequences of loss-of-containment events was explicitly calculated. Events were characterized as high, medium or low frequency (the consequences were calculated for a non-zoonotic virus, so could not be used as a direct comparison). The NEIDL study based their probabilities of accidents on historical incident reports and then assessed risk based on the historical frequency of these accidents and the estimated number of people potentially exposed. Using their data, we characterized every event as high risk (causing more than one human exposure per year), medium risk (causing more than one human exposure per ten years) and low risk (less than one human exposure every ten years).

These previous reports were supplemented by incident/accident reports, including:

- NIH RDAC list of reported incidents, 1977-April 2015 (NIH),
- CDC Select Agent Reports 2003–2009 (CDC—obtained in the appendix of the NEIDL document, above), and

- Various publically available BSL-3&4 accidents (Various—summarized in NEIDL appendix).

From these reports, an incident was characterized as high risk if it represented 10%% or more of accident reports, medium risk if it represented 2% or more of accident reports, and low risk if it accounted for less

14.2 Methodological Details of the Branching Process Model

At the early stages of a nascent outbreak, when a small number of people are infected, stochastic variation between individuals plays a significant role in the eventual size of the outbreak—whether it extinguishes at a small number of cases either due to chance or human intervention, or grows to a large epidemic or global pandemic. For example, if an outbreak begins with a single individual, and this individual self isolates, perhaps due to symptom severity, no other persons may be infected and the outbreak terminates. In contrast, as witnessed in the recent outbreak of MERS in South Korea, a single individual that contacts a large number of people may single-handedly spark a large epidemic. Deterministic models, such as SEIR models, while appropriate for large epidemics in which the number of infected individuals ensures that the mean adequately describes the behavior of most individuals, cannot capture the individual variation inherent in the early stages of an outbreak, and therefore may overestimate the probability that a loss of containment event spreads beyond local control.

In contrast, models that account for this early stochastic variation are more likely to paint an accurate picture of the early phases of a disease outbreak. For this report, a branching process model (BPM) was used in discrete time to capture the individual stochastic variability in nascent outbreaks. Branching process models simulate a "birthing process" over time, and, in the discrete-time branching process model used here, time is represented by generations of infection. Each individual at generation g has a probability of "birthing" (i.e., infecting) a number of offspring (infected individuals) in generation $g+1$ described by a probability distribution (termed the "offspring distribution"). This birthing process is then repeated for a specified number of generations or until some desired stopping point or exit condition is achieved. Typically, the offspring distribution for each individual is the same in each generation, but the distribution can be varied from generation to generation or even from individual to individual, to model, for example, public health control measures. Compared to SEIR models, which model a finite population, branching process models do not consider depletion of susceptible individuals; all outbreaks either self-extinguish or grow indefinitely. As a result, branching process models are only appropriate for the early stages of an epidemic, when the number of infected individuals is negligible compared to the overall susceptible population.

14.2.1 Probability Distribution Used in the Model

This study used an offspring distribution described by a negative binomial distribution with parameters R_0 and k. Negative binomial distributions have been widely studied as models for nascent outbreaks and have been used studying a variety of diseases, including influenza and SARS.[1204,1205] In the distribution, R_0 is the commonly understood mean number of new cases each infected individual generates, and k models the variation between infected individuals. The variable k incorporates differences between individuals caused both by variations in social behavior as well as biological variation in, e.g., shedding or infectious period. At low k values (< 0.5), individual variation is greater, and single individuals have both a greater probability of generating many offspring, as well as a greater probability of generating

[1204] Fraser C *et al* (2011) Influenza transmission in households during the 1918 pandemic. *American journal of epidemiology* 174: 505-514

[1205] Lloyd-Smith JO *et al* (2005) Superspreading and the effect of individual variation on disease emergence. *Nature* 438: 355-359

none. An outbreak described by a small value of k trends toward many outbreaks that self-extinguish rapidly, with a small number of outbreaks that grow rapidly due to one or two individuals generating many offspring. At larger k values, individual variation decreases, with each individual more likely to generate a number of offspring near R_0. A detailed description of the R_0 and k values chosen for influenza, SARS-CoV, and MERS-CoV is provided in the Supporting Information.

14.2.2 Construction of a Two-Type Model for Workers and Community Members

We modeled laboratory workers and community members separately within the BPM. In an outbreak caused by a laboratory loss of control event, laboratory workers and community members are, on average, likely to infect different numbers of individuals due to individual behavior choices, such as self-isolation, as well as potentially different public health control measures and timings of control measures. For example, lab workers are likely to recognize a disease is novel and laboratory-acquired, and may be more likely to self-isolate when infected. Alternatively, if an outbreak is spreading between laboratory workers, the institution or principal investigator may choose to shut down the lab and order isolation of all lab workers, even those not yet showing symptoms, in order to stop the spread. Such drastic steps are unlikely to be possible for entire communities, and community members are less likely to make major changes to daily routines due to infection.

To incorporate these differences and track lab workers and community members separately, we used a two-type branching process model. Two-type branching process models operate similarly to the standard, one-type models, with the modification that the number of offspring distributions is expanded from one to four, for each combination of parent type and offspring type. For a given overall outbreak described by R_0 and k, we modified the single offspring distribution using values described in Table 14.2.

Table 14.2. Modification of Offspring Distribution for Two-Type Model

	Offspring Type			
	Lab Worker		Community Member	
Parent Type	**R_0 value**	**k value**	**R_0 value**	**k value**
Lab Worker	$w*R_0$	k/2	$(1-w)*R_0$	k/2
Community Member	0	k	R_0	k

It was reasoned that a laboratory worker infected by a community member is likely to treat the infection like any regular seasonal or community-acquired disease and not take any special steps to avoid spread, and, additionally, if a laboratory loss-of-containment event has caused an outbreak significant enough that community to community secondary spread is occurring, workers may no longer identify the outbreak as novel or laboratory caused. For this reason, lab workers infected by community members are treated as community members, and the R_0 of community members infected laboratory workers is therefore fixed at zero. As a result, from the perspective of a community member, the branching process model is largely a one-type model, and the offspring distribution for community members is thus properly described by the input R_0 and k values.

For laboratory workers generating both types of offspring, two different probability distributions are used for each type of offspring. The sum of these two distributions (i.e., the probability of generating a total number of offspring) should be equivalent to a negative binomial probability distribution with parameters R_0 and k. The infinite divisibility theorem for negative binomial distribution states that a negative binomial distribution, O, described by parameters R_0 and k, can be broken into n independent negative binomial distributions with parameters R_0/n and k/n. For two distributions, W and C would each be

negative binomial distributions described by $R_0/2,k/2$, O = W+C, where O describes the distribution describing the total number of offspring, W describes the offspring distribution for workers generating workers, and C describes the offspring distribution for workers generating community members.

Distributions W and C, as described above, have identical parameters, and thus workers would be equally likely to generate workers and community members. However, workers are more likely to contact their colleagues in the workplace than they are community members, and thus are more likely to infect additional workers than community members. Based on a survey of individual contact frequencies containing data on the location of the contact,[1206] the fraction of contacts that laboratory workers would have in the workplace versus elsewhere was estimated, and incorporated this into a parameter, w, where w is the fraction of contacts that occur at work and (1-w) is the fraction that occur elsewhere. The R_0 values of the corresponding two-type offspring distributions are then multiplied by these factors. For the infinite divisibility theorem to hold, the R_0 values of each of the two-type offspring distributions must be equal, and each half of the one-type distribution. However, for values of w near 0.5, the error between the one-type distribution and sum of the two-type distribution (W+C-O) is small, where low numbers of offspring are slightly less likely and large numbers of offspring slightly more likely in the two-type model, with a maximum error of any specific offspring number of approximately 0.5%. On average, the total number of offspring generated is slightly less for the sum of the two-type distributions than the one type distribution but the difference is typically <0.5% of the total number of offspring generated, though statistical fluctuations can result in more total offspring being generated by the sum of two-type distributions. Figure 14.2 illustrates this difference for one million draws from each distribution, with R_0= 1.3, k=1.0, values appropriate for seasonal flu, and w =0.6951, the value used in all of our simulations.

[1206] Mossong J *et al* (2008) Social contacts and mixing patterns relevant to the spread of infectious diseases. *PLoS medicine* 5(e74

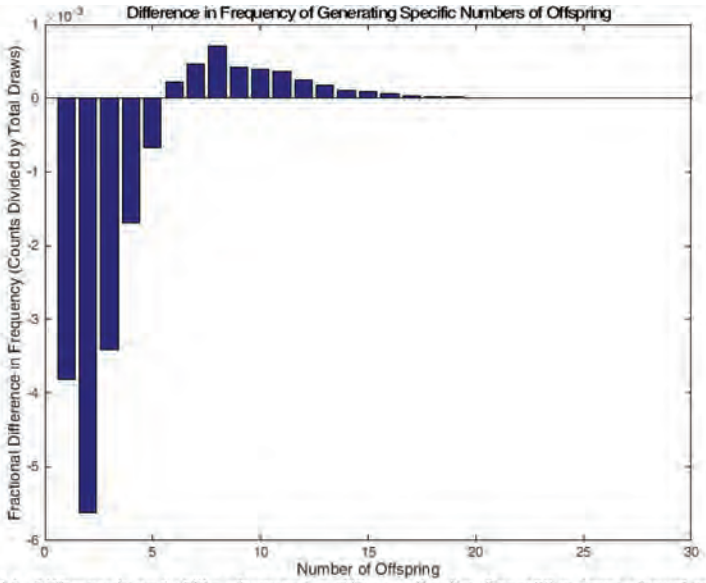

Figure 14.2. Difference in probability of generating different offspring. One million draws of number of offspring were done for the one-type and each two-type distribution. The y-axis shows the difference between the distributions as a fraction of the total number of draws.

14.2.3 Incorporation of Control Measures

Two types of control measures were incorporated into the model: population-wide control (i.e., social distancing) and individual control (quarantine and isolation), based on previous work by Lloyd-Smith and colleagues.[1207] In the model used in this project, these types of control measures can be active in one of five possible combinations as summarized by Table 14.3. We did not consider population-wide control on laboratory workers in the absence of control on community members, because social distancing on just a small section of the population is unprecedented and unwarranted. Additionally, individual control on the community in the absence of control on laboratory workers was not considered, as it did not seem reasonable to quarantine some individuals while avoiding quarantine on those most likely to be infected.

[1207] Lloyd-Smith JO *et al* (2005) Superspreading and the effect of individual variation on disease emergence. *Nature* 438: 355-359

Table 14.3. Control Measure Combinations		
Number	**Control on Laboratory Workers**	**Control on Community Members**
1	Individual	None
2	Individual	Individual
3	Population	Population
4	Individual & Population	Population
5	Individual & Population	Individual & Population

Each control measure, is implemented by modifying the corresponding offspring distribution via two parameters: *c*, representing the strength of control, which vary from zero (no control) to one (perfect or absolute control), and *g*, the generation at which control becomes active, with the modifications to the offspring distribution only present if the current generation is greater than or equal to *g*. In our model, the control strengths of the two types of control may vary independently of each other, but the strength of a particular control measure is the same for lab workers and community members. This reasoning was based on the idea that public health and other responders to a nascent outbreak would be unlikely to, for example, loosely quarantine lab workers and tightly control community members, or vice versa. (Recall that the early stages of the response to loss-of-containment events on laboratory workers takes place within the worker response event tree prior to the initiation of the BPM) The modifications to the offspring distribution for each type of control are summarized in Table 14.4.

In population wide control, each individual reduces the number of contacts they have by a fraction given by the control strength c_p, resulting in a decrease in the mean number of people a person infects, and thus, R_0, by a factor $(1-c_p)$. However, the variation between individual's infectiousness does not change, resulting in the same k value prior to control being active.

In the type of individual control modeled here, a fraction, c_i, of those infected individuals that would have otherwise generated a non-zero number of offspring are isolated and thus instead generate none. This measure has the net effect of increasing the proportion of zeros in the resulting offspring distribution. As discussed in work by Lloyd-Smith and colleagues,[1208] modeling of this control measure can be accomplished in one of two ways: by directly modifying the resultant draws from the negative binomial distribution and, with probability c_i, setting the number of offspring for draws greater than zero to zero, or by finding a solution to an alternative analytical equation, the solution to which gives a an approximate k value assuming a negative binomial distribution, resulting in a negative binomial distribution with parameters $(1-c_i)*R_0$ and k_i that closely resembles the exact distribution under control for almost all of k space.[1209] In the former approach, the control measure is modeled exactly, but the effective value of k used is not-knowable *a priori*. In the latter, the control measure is modeled with some error, but the exact value of effective k is known, as it is specified in the negative binomial draws done under control. In the approach used here, the latter approach was taken because computing the probability an outbreak self-extinguishes requires knowing a value for k.

[1208] Ibid.
[1209] Ibid.

Table 14.4. Modification to the Offspring Distribution for Control Measure Types

		Offspring Type			
		Lab Worker		Community Member	
Parent Type	**Control Measure**	**R_0 Value**	**k value**	**R_0 Value**	**k value**
Lab Worker	Individual	$(1-c_i)*w*R_0$	$k/2$	$(1-c_i)*(1-w)*R_0$	$k_i/2$
	Population	$(1-c_p)*w*R_0$	$k/2$	$(1-c_p)*(1-w)*R_0$	$k/2$
	Both	$(1-c_i)*(1-c_p)*w*R_0$	$k_i/2$	$(1-c_i)*(1-c_p)*(1-w)*R_0$	$k_i/2$
Community Member	Individual	0	k_i*	$(1-c_i)*R_0$	k_i
	Population	0	k	$(1-c_p)*R_0$	k
	Both	0	k_i	$(1-c_i)*(1-c_p)*R_0$	k_i

**As R_0 for community members generating workers is 0, the value of k has no effect on the resulting distribution*

14.2.4 Terminating Models Due to Loss of Control

As mentioned in the main text, the BPM was used to model the initial stages of an outbreak, when it is still circulating in the local community and has the potential to self-extinguish or be brought under control. The simulations of each outbreak were terminated when one of four conditions was met:

- The outbreak self-extinguished (no new cases were generated by any infected individuals in the current generation).
- Beginning in generations after all control types, if any, had been activated, the model calculated that, given the number of cases in the current generation, that the outbreak had less than a 5% chance of extinguishing at any point in the future.
- That any generation included 1,000 or more infected individuals.
- The outbreak had persisted for 200 generations without any of the above conditions being met.

The outbreak was considered out-of-control when any of the conditions other than self-extinguishing was met. An outbreak that had less than 5% chance of self-extinguishing even after all control measures were implemented was highly likely to grow to a size beyond that which local health officials could contain. Even if an outbreak had a significant chance of self-extinguishing, 1000 simultaneous cases would likely overwhelm the capacity of local resources to contain, and outbreaks would likely seed elsewhere prior to when the outbreak self-extinguished. Finally, outbreaks were terminated after 200 generations to avoid wasting a significant fraction of computational resources on simulations that, by stochastic chance, persist for considerable lengths of time without meeting any other termination condition. This condition was never reached in any simulation for 97.7% of the more than five million parameter combinations tested and reached less than 5% of the time in 99.5% of parameter combinations tested, thereby having an insignificant effect on the results. Because the outbreak had still not self-extinguished, these outbreaks were considered out-of-control in order to conservatively estimate risk. However, should an outbreak persist for 200 generations within a local community, travel of individuals in and out of the community would result in a high likelihood of the outbreak seeding elsewhere, representing a local loss-of-control event.

14.2.5 Calculation of Self-Extinguishing Probability

In a two-type branching process model with 2x2 matrices of R_0 and k values, where $R_{0,ij}$ and k_{ij} represent the values for type i individuals generating type j offspring, the probabilities of the outbreak self-extinguishing at some future generation given one infected individual of each type in the current generation, $[q_1\ q_2]$, where q_1 is the probability of an outbreak with one individual of type 1 and q_2 the same for a type 2 individual, can be derived from the basic principles of branching process models,[1210] assuming $R_{0,ij}$ and k_{ij} are time-invariant, with $[q_1\ q_2]$ as solutions for of the following system of equations:

$$\left(1+\left(\frac{R_{11}}{k_{11}}*(1-q_1)\right)\right)^{-k_{11}}*\left(1+\left(\frac{R_{12}}{k_{12}}*(1-q_2)\right)\right)^{-k_{12}}-q_1=0$$

$$\left(1+\left(\frac{R_{21}}{k_{21}}*(1-q_1)\right)\right)^{-k_{21}}*\left(1+\left(\frac{R_{22}}{k_{22}}*(1-q_2)\right)\right)^{-k_{22}}-q_2=0$$

Given the values of q_1 and q_2, and infected numbers of individuals I_1 and I_2, the overall probability of the outbreak self-extinguishing is given by:

$$q_{tot}={q_1}^{I_1}*{q_2}^{I_2}$$

As the equations solved presume time-invariance in R_0 and k, the outbreak was not presumed out of control until $q_{tot} \leq 0.05$ at a generation after all control measures, and therefore modifications to R_0 and k had taken place.

14.3 Methodological Details of the HHS-BARDA IIM

14.3.1 Computation of Region-Specific Contact Rate Matrices

Each of the twelve global regions simulated by the HHS-BARDA Interactive Influenza Model (referred to as the IIM) used a different contact matrix that incorporated demographic differences between regions, including age distribution, household size distribution, and school class size. For the region representing high income countries in Europe and Central Asia (ECA), we used primary data gathered in a contact survey of several countries within that region[1211] to calculate a 4x4x6 three-dimensional matrix of mean contact frequencies, F, where f_{ijk} is the expected daily number of contacts a person in age bracket i and living in household size k would make with people in age bracket j. Reported contacts where either the age of the reporter or person contacted, or household size of the reporter were unknown or omitted were removed from the data set. Persons were grouped into four age groups: 0-4 years old, 5-19, 20-64, and 65+, representing young children and infants, school-aged children, adults, and the elderly, respectively, based on the default age groups used by BARDA. Households were divided into size 1-5, and 6+, based on the groupings in the primary data. For each individual reporting contacts, the sum of the number of contacts that individual made with persons of each age group were tracked and summed with other individuals of the same age and household size, to create a 4x4x6 matrix of total number of contacts by age and household size. This matrix was divided by the number of reporters within each age group and household size to get the mean contact frequency matrix F.

[1210] For a review of branching process models see Harris TE (2002) *The theory of branching processes*: Courier Corporation.

[1211] Mossong J *et al* (2008) Social contacts and mixing patterns relevant to the spread of infectious diseases. *PLoS medicine* 5: e74

This 4x6x4 matrix was reduced to a 4x4 matrix C, where c_{ij} represents the daily frequency of contact of an individual of age i with persons of age j using a population weighted average of the per-household size contact rates, using the following equation:

$$c_{ij} = \sum_{k=1}^{6} f_{ijk} * h_k$$

where h_k represents the fraction of people living in a household of size k.

For high income ECA, the matrix C, after balancing (see below), was used as the contact matrix in the IIM simulations for that region. For the other regions, the same matrix F was combined with region-specific h_k values to generate a new matrix C. In addition, each other matrix was modified to account for local differences in population distribution by age and class size compared to high income ECA. We assumed that the age-specific contact rates, c_{ij} vary proportionally to the fraction of the population of age j (i.e., the more individuals of a certain age composing a community, the larger the frequency any individual would contact one of them), by the following equation:

$$c_{ij} = b_{ij} * a_j$$

where a_j is the fraction of people in the region simulated of age j, and b_{ij} is a scalar multiplier that remains fixed across all regions. Using the matrix C for high income ECA, presumed to already be corrected by the scalars b, corrected c values for other countries were calculated using the following relationship (and using North America, abbreviated NA, as an example):

$$c_{ij,ECA}/c_{ij,NA} = b_{ij*a_{j,ECA}}/b_{ij*a_{j,NA}} \Rightarrow c_{ij,NA} = c_{ij,ECA}{}^{a_{j,NA}}/a_{j,ECA}$$

Similarly, the contact frequency of school age children contacting school age children was corrected using a class-sized based multiplier. We assumed that the contact rate of children with children, c_{22}, varied by:

$$c_{22} = d * s$$

where s is the average class size within a region and d is a scalar multiplier again fixed across all regions. Using a similar relationship to that of the age-specific correction above, the correction for class size becomes:

$$c_{22,NA} = c_{22,ECA}{}^{s_{NA}}/s_{ECA}$$

Each of these computations of c_{ij} were presumed to be multiplicative and independent such that, using the rate of children contacting children in North America as an example, the overall calculation of c_{22} becomes:

$$c_{22,NA} = \left(\sum_{k=1}^{6} f_{22,k} * h_{k,NA}\right) * a_{j,NA}/a_{j,ECA}{}^{*} s_{NA}/s_{ECA}$$

In addition to the corrections made above, each matrix C was also balanced. Given that every contact involves two individuals, the total number of contacts all people of age i make with age j must also equal the number of contacts people of age j make with age i. This can be represented in the contact matrix by:

$$c_{ij} * a_i = c_{ji} * a_j$$

Because the primary data contained no information on how to correct for any apparent imbalances in overall contact rates, the matrices were balanced by assuming the overall contact numbers were equal to the mean contact numbers of people of age i with age j and j with i. To accomplish this with each matrix C, each element of the matrix, c_{ij}, was multiplied by the fraction a_i of the population of that age to result in a new matrix of proportional contact numbers D. That matrix was added to its transpose D^T and then each element was divided by two to get a balanced matrix of mean proportional contact numbers. Finally, each element of the matrix was divided by the fraction a_i of the population to convert the balanced contact number matrix into a balanced contact rate matrix C_B, where each element of C_B is given by the following equation:

$$c_{B,ij} = \left(c_{ij} * a_i + c_{ji} * a_j\right) / (2a_i)$$

These balanced contact rate matrices were used as inputs into the IIM.

14.4 Additional Data on the Potential Proliferation of GoF Research

Table 14.5. Terms Used to Query PubMed and Web of Science Databases.
[enhanced or increased] and [morbidity or mortality or pathogenicity] and [influenza virus]
[increased or enhanced] and [virulence] and [influenza virus]
[increased or enhanced] and [tropism] and [influenza]
[increased] and [human or mammalian] and [adaptation] and [influenza]
[immune system evasion] and [influenza]
[increased or enhanced] and [transmission] and [influenza virus]
[increased infectivity] and [influenza virus]
[h1n1] and [increased or enhanced] and [transmission or virulence or immune evasion or tropism or mortality or morbidity or infectivity]
[h1n9] and [gain of function]
[h7n9] and [enhanced or increased] and [transmissibility or tropism or mortality or morbidity or viral production or resistance or immune evasion]
[sars or severe acute respiratory syndrome]
[enhanced or increased] and [morbidity or mortality or pathogenicity] and [sars or mers]
[enhanced or increased] and [virulence] and [sars or mers]

Table 14.5. Terms Used to Query PubMed and Web of Science Databases.
[enhanced or increased] and [tropism] and [sars or mers]
[enhanced or increased] and [human or mammalian adaptation] and [sars or mers]
[enhanced or increased] and [immune system evasion] and [sars or mers]
[enhanced or increased] and [transmission] and [sars or mers]
[enhanced or increased] and [infectivity] and [sars or mers]

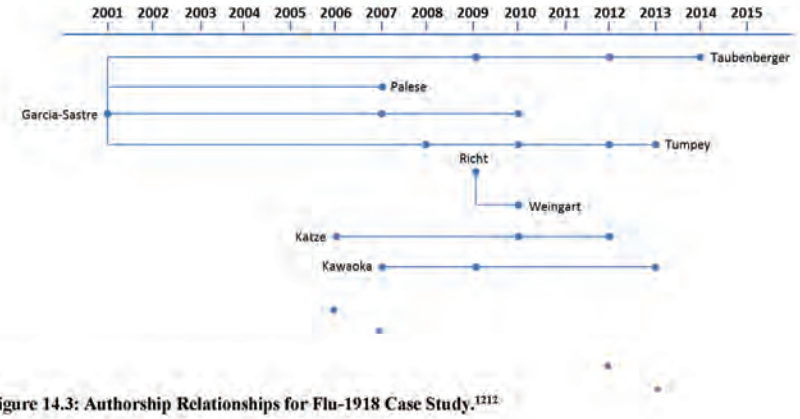

Figure 14.3: Authorship Relationships for Flu-1918 Case Study.[1212]

[1212] Each dot represents a paper with an indicated last author. If an earlier middle author became a last author on a subsequent paper with a different last author, a line was drawn between the dots.

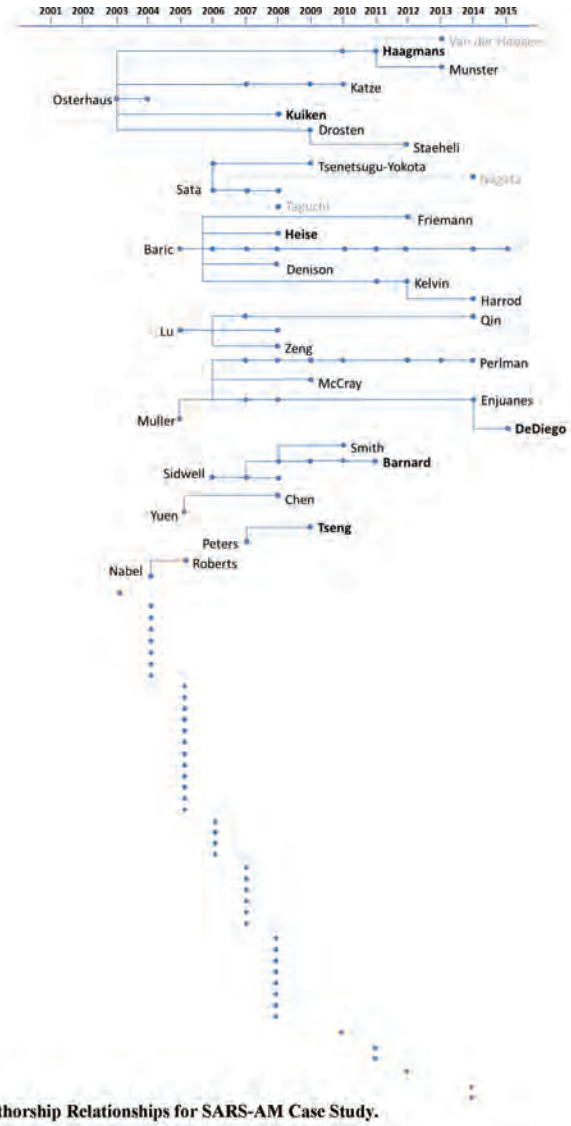

Figure 14.4: Authorship Relationships for SARS-AM Case Study.

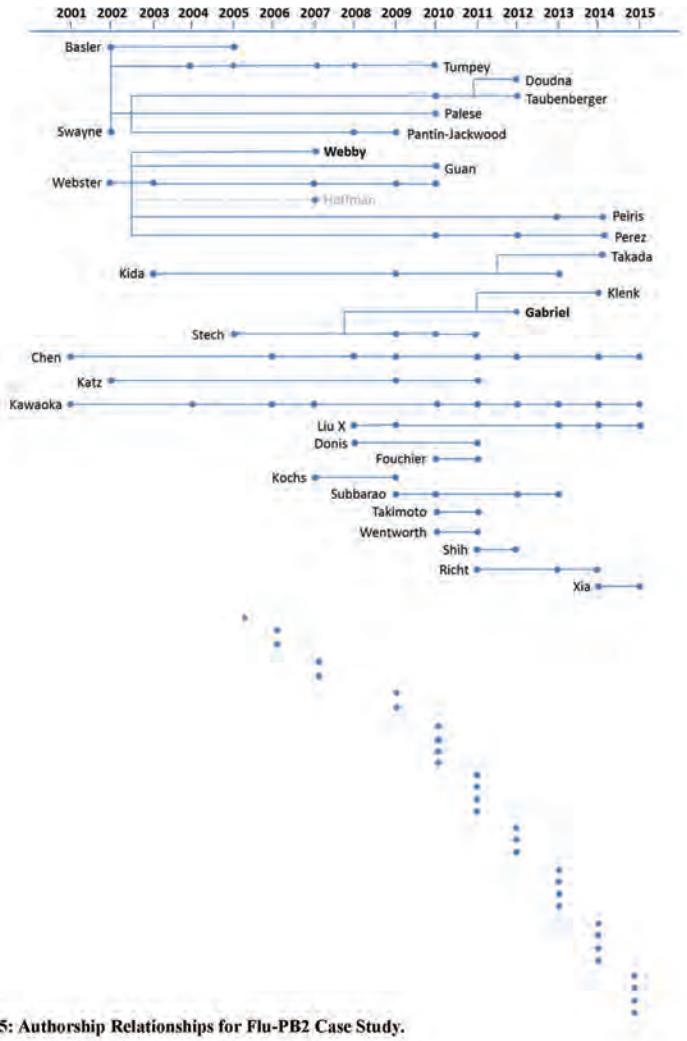

Figure 14.5: Authorship Relationships for Flu-PB2 Case Study.

15 Appendix IV. Benefit Assessment

Chapter 15 provides fully referenced, in-depth discussions of the potential benefits of GoF research involving coronaviruses and influenza viruses. An overview of these benefits is provided in chapter 9.3 through 9.11 and 9.14.

15.1 Coronaviruses: Detailed Analysis of the Benefits of GoF Research

15.1.1 Introduction

15.1.1.1 Scope of Assessment

This assessment describes the benefits of GoF experiments involving SARS-CoV, MERS-CoV, and SARS/MERS-like bat CoVs. From a review of the coronavirus literature, experimental approaches that are reasonably anticipated to lead to the following phenotypic changes were identified:

- Enhanced pathogen production as a result of changes in the replication cycle or growth,
- Altered host range (typically accompanied by enhanced virulence in the new host),
- Enhanced fitness or virulence in cell culture or laboratory animal model systems, respectively and
- Evasion of therapeutics in development.

As current animal models for studying coronaviruses do not support transmission between animals, this field does not include any approaches that lead to enhanced transmission in appropriate animal models. Additionally, because there is no widespread population immunity to the coronaviruses and there are no licensed coronavirus vaccines, this field does not include any approaches that lead to evasion of existing natural or induced immunity. Finally, no coronavirus research that is reasonably anticipated to lead to evasion of diagnostics or of vaccines in development was identified. (It should be noted that there are currently no FDA-approved vaccines or therapeutics for coronaviruses.)

The four human coronaviruses that cause mild to moderate respiratory illnesses such as the common cold or croup (coronaviruses HKU1, OC43, 229E, and NL63) were not evaluated because these are not considered in the NSABB GoF Framework. Throughout this report, use of the term "coronaviruses" or "CoVs" refers specifically to SARS-CoV, MERS-CoV, and SARS/MERS-like bat CoVs such as HKU4 and HKU5.

15.1.1.2 Overview of Coronavirus GoF Landscape

Here, a brief overview of the experimental approaches within each GoF phenotypic category is provided. Each approach will be discussed in more detail in the context of detailed analysis of the benefits of GoF research involving coronaviruses, below.

15.1.1.2.1 Experimental Approaches That Lead to Enhanced Pathogen Production

Serial passaging of CoV in cell culture leads to the generation of higher-yield viruses. This approach is used to enhance the growth of viruses with naturally poor growth properties, in order to develop an *in vitro* model system for experimental use.

15.1.1.2.2 Experimental Approaches That Alter Host Tropism in Mammals

Several experimental approaches alter the host range of CoVs. One approach involves "Spike swapping" – that is, targeted genetic modification to replace all or part of the coronavirus Spike protein, a viral surface protein that mediates virus entry into cells and is a critical determinant of host restriction, with the Spike protein from another CoV species. This manipulation leads to the generation of a recombinant, chimeric CoV that may exhibit altered host tropism relative to the parental CoV species. The purpose of these experiments is three-fold:

- Introducing the SARS Spike protein into the backbone of bat CoVs, which do not efficiently infect standard cell culture lines or animals, enables the chimeric virus to infect cells/animals, thus creating a tool that can be used to study the biology of the bat CoV,

- Chimeric viruses are used as tools to test whether CoV therapeutics and vaccines are broad-spectrum, capable of protecting against potentially emerging SARS/MERS-like bat CoVs as well as SARS and MERS, and

- Testing the ability of chimeric CoVs to infect various types of cells and animals reveals the breadth of host tropism conferred by a given Spike protein, and comparing the sequences of parental and donated Spike proteins with different host tropism can uncover amino acid residues that mediate host restriction.

A second approach that leads to altered host range involves serial passaging of CoVs in mice, which leads to the generation of viruses that have adapted to more efficiently infect and cause disease in mice. The purpose of this experiment is two-fold:

- Mouse-adapted strains are experimental tools that are used for the study of disease pathogenesis and for testing the efficacy and safety of vaccines and therapeutics, and

- Comparing the sequences of the mouse-adapted and the parental strain leads to the identification of mutations that are associated with adaptation, which provides a foundation for follow-up studies investigating the mechanistic basis of virus adaptation to new hosts.

A final approach involves targeted mutagenesis to introduce mutations that are associated with altered host tropism, which is performed to demonstrate that the mutation(s) are necessary and sufficient to alter host tropism. As above, this information provides a foundation for follow-up studies investigating the phenotypic traits underlying virus adaptation to new hosts.

15.1.1.2.3 Experimental Approaches That Enhance Fitness or Virulence in Cell Culture or Laboratory Animal Model Systems

Several experimental approaches enhance the fitness or virulence of CoVs in cell culture or laboratory animal model systems, respectively. First, serial passaging of CoVs in mice leads to the generation of viruses with both enhanced infectivity to and virulence in mice. Because of the specificity of virus-host interactions that are important determinants of host tropism and pathogenicity, this adaptation often translates to reduced virulence in humans. The purpose of this experiment is two-fold:

- Enhancing the virulence of the virus in mice is an important aspect of creating a mouse model that replicates human disease pathology, which is needed for the study of disease pathogenesis mechanisms and the testing of medical countermeasures, and

- Comparing the sequences of the mouse-adapted and the parental strain leads to the identification of mutations that are associated with enhanced virulence, which provides a foundation for follow-up studies to elucidate the mechanistic basis of virulence. This information can also benefit public health by identifying new potential targets for therapeutics or for attenuation, in order to create attenuated vaccine viruses.

A second approach involves targeted genetic modification of viruses to introduce mutations that are associated with enhanced virulence, which is performed to demonstrate that the mutation(s) are necessary

and sufficient to enhance virulence. As above, this information provides a foundation for follow-up studies to elucidate the mechanistic basis of virulence.

A third approach involves serial passaging of attenuated viruses that are candidate live attenuated vaccines (LAVs), in order to determine whether the viruses acquire mutations that enhance fitness/virulence. Because LAVs with an ability to recover fitness during growth *in vivo* could cause adverse outcomes in people, a negative result is an important indicator of safety for any live attenuated vaccine in development.

15.1.1.2.4 Experimental Approaches That Lead to Evasion of Therapeutics in Development

Serial passaging of a virus in cells in the presence of a therapeutic may lead to the emergence of viruses that are resistant to inhibition/neutralization by that therapeutic. The purpose of the experiment is to understand whether and how readily resistance will arise in response to selective pressure from the therapeutic and to identify mutations that are associated with resistance to the therapeutic, which provides a foundation for follow-up studies investigating the mechanisms underlying antiviral activity and antiviral resistance. This information benefits the development of these therapeutics. Specifically, emergence-of-resistance data speak to the potential field efficacy of the therapeutic, and information on both antiviral mechanism and emergence of resistance are important components of an investigational new drug application to the FDA.

15.1.2 Overview of the Potential Benefits of GoF Experiments Involving Coronaviruses

This section evaluates whether any of the GoF CoV approaches have the potential to benefit each of the general benefit areas described in the NSABB's "Framework for Conducting Risk and Benefit Assessments of Gain of Function Research." Also described are additional benefit areas identified during research. Each potential benefit will be analyzed in detail below.

15.1.2.1 Scientific Knowledge

GoF approaches have the potential to directly benefit scientific knowledge by providing insight into the mechanisms underlying adaptation of coronaviruses to new hosts as well as the mechanistic basis of coronavirus virulence. In addition, the development of animal models using GoF approaches has the potential to indirectly benefit scientific knowledge by enabling the study of disease pathogenesis, including the role of host factors in disease pathology.

15.1.2.2 Surveillance

Currently, GoF approaches do not have the potential to benefit public health, agricultural animal, or wildlife surveillance. Although CoV researchers stated that they could envision using information about the molecular determinants of human adaptation and virulence to assess the risk posed by animal CoVs circulating in nature, similar to the influenza field, this application is currently unfeasible for two reasons: (1) CoV surveillance networks are extremely limited, with large gaps in coverage in humans and animals, and (2) the state of knowledge about the molecular determinants of human adaptation and virulence is poor.[1213]

[1213] For example, out of more than 1700 bat species, only ten have been surveilled for evidence of CoV infection (and those ten on an ad hoc rather than a systematic basis).

15.1.2.3 Vaccines

GoF approaches have the potential to benefit the development of vaccines in three ways:

- GoF approaches that lead to the discovery of virulence factors identify potential gene targets for attenuation, for the development of live attenuated vaccines (LAVs),
- Serial passaging of LAV strains in animals is used to test whether strains recover virulence upon growth *in vivo*, which is an important aspect of vaccine safety, and
- GoF approaches that lead to the development of animal-adapted viruses (i.e., serial passaging of viruses in laboratory animals to alter host tropism and enhance virulence) enable the testing of vaccine candidates in animal models that mimic the pathology of human disease.

15.1.2.4 Therapeutics

GoF approaches have the potential to directly benefit the development of therapeutics in several ways:

- GoF approaches that lead to the discovery of virulence factors identify potential new therapeutic targets,
- GoF approaches that lead to evasion of therapeutics in development provide information about the potential field efficacy of the therapeutic and the mechanism of activity of the therapeutic, both of which are critical components of an Investigational New Drug application to the FDA,
- GoF approaches that lead to evasion of therapeutics in development can provide insight into the therapeutic dosing regimens and combination therapies (e.g., cocktails of monoclonal antibodies) that are the least likely to permit evolution of resistance, and
- GoF approaches that lead to the development of animal-adapted viruses enable the testing of therapeutic candidates in animal models that mimic the pathology of human disease.

15.1.2.5 Diagnostics

As diagnostic targets for CoVs are well-established, no potential benefits of GoF approaches to the development of diagnostics were identified.[1214],[1215],[1216],[1217]

[1214] The FDA-approved diagnostic test for MERS-CoV targets two regions in the CoV genome: a region upstream of the E gene (*upE*) and the reading frame 1a (*orf1a*). SARS can be detected through RT-PCR with sequences in the polymerase 1 B region (*pol* 1B) and an adjacent downstream region of the genome as the targets. Other diagnostic tests target sequences in the nucleocapsid (N) gene.

[1215] Stephen M. Ostroff Acting Commissioner of Food and Drugs. Letter of Authorization RealStar® MERS-CoV RT-PCR Kit U.S. . http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM455348.pdf. Last Update July 17, 2015. Accessed December 2015.

[1216] Richardson SE *et al* (2004) The laboratory diagnosis of severe acute respiratory syndrome: emerging laboratory tests for an emerging pathogen. *The Clinical biochemist Reviews / Australian Association of Clinical Biochemists* 25: 133-141

[1217] Mahony JB *et al* (2004) Performance and Cost evaluation of one commercial and six in-house conventional and real-time reverse transcription-per assays for detection of severe acute respiratory syndrome coronavirus. *J Clin Microbiol* 42: 1471-1476

15.1.2.6 Informing Policy Decisions

Because the US government is not actively engaged in public health preparedness activities for CoV outbreaks and because there are no FDA-approved vaccines or therapeutics for CoVs, GoF approaches do not have the potential to benefit decision-making in public health policy (e.g., informing countermeasure stockpiling decisions, guiding decisions about strain selection for vaccine development, etc.)

15.1.2.7 Economic Benefits

GoF benefits to the development of vaccines and therapeutics could have downstream economic benefits. Economic benefits were not explicitly evaluated in this report.

Below the potential benefits in all the fields identified above are analyzed: scientific knowledge, vaccines, and therapeutics. For each field, the potential benefits of GoF approaches as well as the potential benefits of alternative experimental approaches and alternative scientific and technical innovations that can provide the same or similar benefits are analyzed. For each potential benefit, the scientific, technical, and regulatory barriers to the realization of that benefit were identified; these impact the likelihood and timing of the realization of the benefit. Next, the potential benefits of GoF research relative to alternative approaches are evaluated, considering the barriers to the realization of the benefits of each.

This analysis is split into three sections. First, the potential for GoF approaches to directly benefit scientific knowledge, including knowledge about mechanisms underlying the cross-species adaptation and pathogenesis of coronaviruses, is evaluated. In this section, alternative experimental approaches that can provide the same or similar information as GoF approaches are considered. Second, the potential benefits of using model systems developed using GoF approaches are analyzed; these include benefits to basic science research as well as to medical countermeasure (MCM) development. In this section, alternative model systems that do not involve GoF approaches are evaluated (e.g., use of a naturally susceptible host in lieu of using a virus adapted to a laboratory animal). Finally, the potential for GoF approaches to directly benefit public health is assessed; this includes benefits to the development of vaccines and therapeutics. In this section, alternative experimental approaches as well as alternative scientific and technical innovations that have the potential to similarly benefit MCM development are evaluated.

15.1.3 Benefits of GoF to Scientific Knowledge

Several GoF approaches generate information that directly benefits scientific knowledge by providing insight into critical unanswered questions about coronavirus biology. Specifically, GoF approaches that alter host tropism can provide insight into the mechanistic basis of cross-species adaptation, and GoF approaches that enhance virulence in animal models enable the identification of virulence factors and deepen understanding of the mechanisms underlying pathogenicity. In this section, the benefit of GoF approaches to each of these scientific areas, relative to alternative experimental approaches that can provide the same or similar scientific information, are discussed.

15.1.3.1 Scientific Knowledge Gap 1: How Do Animal Coronaviruses Adapt to Humans? What Are the Genetic and Phenotypic Traits Underlying Adaptation to Humans?

SARS and MERS unexpectedly emerged from their animal reservoirs to infect humans in 2002 and 2012, respectively. Surveillance of bats and other CoV reservoir species indicates that there is a large diversity of animal CoVs circulating in nature, including many species that are genetically related to SARS and

MERS and thus may have the potential to spill over into human populations in the future.[1218,1219,1220,1221] Although multiple coronaviruses have been shown to exhibit a flexible capacity for cross-species transmission,[1222,1223] the mechanisms underlying CoV adaptation to new host species are poorly understood. Specifically, large gaps in knowledge remain regarding:

- The mechanistic basis of cross-species adaptation – what viral factors are involved, and what phenotypic changes must occur in order for a CoV to adapt to efficiently infect and cause disease in a new host species?
- The evolutionary mechanisms driving cross-species adaptation – what selective pressures drive adaptation to new host species, and what is the order of acquisition of new genetic/phenotypic traits needed for adaptation? And
- Whether the ability to adapt to new species is a conserved feature of all CoVs, and if so, whether the mechanisms underlying adaptation of different CoV species are similar or distinct?

15.1.3.1.1 Potential Benefits and Limitations of GoF Approaches

Several GoF approaches can provide insight into these questions. Serial passaging of CoVs in cells derived from a non-natural host organism or in a non-natural laboratory animal host selects for viruses that more efficiently infect cells/animals, thereby enabling the identification of mutations that are sufficient for adaptation to a new host species. Currently in the CoV field, these experiments involve passaging of animal or zoonotic CoVs (such as MERS-CoV) in human cells or passaging of MERS-CoV in mice. (SARS-CoV was previously adapted for growth in mice through serial passaging.) Identifying where mutations arise during adaptation to new hosts points to viral factors that may play a role in adaptation, and studying the phenotypic consequences of the mutations provides insight into the mechanistic basis of cross-species adaptation. Of note, serial passaging in simple, *in vitro* model systems provides more limited information about mechanisms underlying cross-species adaptation than serial passaging in animals, and the phenotypic changes needed to adapt viruses for growth in cell culture may not be relevant for *in vivo* adaptation. Analyzing viral sequences at multiple stages of *in vivo* passaging can provide insight into the order of acquisition of genetic changes as well as information about mutations that are positively and negatively selected over the course of adaptation. One key benefit of this approach is that it can lead to the discovery of novel genetic traits and virus proteins that are involved in the process of adapting to new hosts without the need for prior knowledge of viral adaptation factors. Moreover, this approach can be used to explore the adaptation of any virus to a new host species, provided that the virus can be grown in an appropriate model system. Finally, repeating the serial passaging experiment multiple times with the same starting virus can provide insight into the mutational landscape of cross-species adaptation – that is, whether the same changes tend to occur or whether there are multiple evolutionary pathways for adapting to a new host species. The main limitations of this approach are that traits that promote growth in a particular cell type or a non-human mammal may not be required for enhancing the

[1218] Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology* 84: 3134-3146

[1219] Yang Y *et al* (2015) Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. Ibid. 89: 9119-9123

[1220] Pfefferle S *et al* (2009) Distant relatives of severe acute respiratory syndrome coronavirus and close relatives of human coronavirus 229E in bats, Ghana. *Emerging infectious diseases* 15: 1377-1384

[1221] Ge XY *et al* (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538

[1222] Baric RS *et al* (1999) Persistent infection promotes cross-species transmissibility of mouse hepatitis virus. *Journal of virology* 73: 638-649

[1223] Chen W *et al* (2005) SARS-associated coronavirus transmitted from human to pig. *Emerging infectious diseases* 11: 446-448

ability of the virus to infect and transmit between humans and that laboratory evolution may not mimic natural selection. Additionally, serial passaging identifies traits that are sufficient but may not be necessary for adaptation to new hosts, and results gleaned from the one or two strains under study may not be conserved in other CoV species.

Another GoF method for studying cross-species adaptation involves "Spike swapping" – that is, targeted genetic modification to replace all or part of the CoV Spike protein, a surface protein that mediates virus entry into cells and is a critical determinant of host restriction, with the Spike protein from another CoV species. These experiments are considered Gain of Function because they are expected to alter host tropism in mammalian species. The purpose of these experiments is two-fold. First, testing the ability of chimeric CoVs to infect various types of cells and animals reveals the breadth of host tropism conferred by a given Spike protein, and comparing the sequences of parental and donated Spike proteins with different host tropism can uncover amino acid residues that mediate host restriction. Second, defining the host tropism of animal CoVs and the number of amino acid changes that are needed to confer the ability to infect human cells provides insight into whether the ability to adapt to new species is a conserved feature of CoVs, as well as which animal CoVs are poised to spill over into human populations. (Of note, these high-risk bat CoVs can then be targeted as part of efforts to develop broad-spectrum vaccines and therapeutics, which will be discussed further in Section 16.1.4.) The main drawback of this approach is that it is limited to studying the role of the Spike-receptor interaction, and no other viral factors, in host tropism. Another drawback is that chimeric "SARS plus animal CoV Spike" viruses may behave differently from wild type animal CoVs; however, presenting an animal CoV Spike in the context of the SARS virus better mimics the wild type virus than pseudotyping systems using other viruses, an alternative approach discussed below. (Pseudotyping is the process of expressing the envelope protein or surface glycoprotein from one virus on the surface of a different virus, e.g., replacement of the vesicular stomatitis virus glycoprotein (VSV G) with the CoV Spike, enabling expression of the CoV Spike on the surface of VSV. Pseudotyping is performed to study the function of the foreign virus protein in isolation, as a risk mitigation measure, and/or to study the activity of a protein from a virus that is difficult to culture, such as bat CoVs.)

Finally, targeted genetic modification of wild type viruses to introduce mutations that are associated with adaptation to new hosts demonstrates that such markers are *necessary* and *sufficient* to broaden or alter host tropism. Of note, these mutations can be discovered through a GoF approach, such as serial passaging, or an alt-GoF approach, such as comparative sequence analysis (discussed below). This information provides a strong foundation for follow-up studies investigating the mechanistic basis of the adaptation phenotype.

15.1.3.1.2 Potential Benefits and Limitations of Alt-GoF Approaches

Alternative experimental approaches can also be used to discover genetic traits associated with cross-species adaptation of CoVs. First, comparing the sequences of CoVs with different species tropism, including comparison of animal CoVs versus SARS/MERS and comparison of animal strains from different geographic regions where spillover into human populations has and has not occurred (or has occurred with different frequencies), can elucidate genetic traits that are associated with adaptation to different hosts. Second, comparative sequence analysis of human CoVs from different time points during an outbreak reveals how zoonotic CoVs adapt to humans following an initial spillover event. Relative to the laboratory methods described above, this approach may be more likely to uncover conserved determinants of cross-species adaptation because it involves analysis of multiple sequences, and analysis of human isolates is more likely to identify traits that are relevant for adaptation to humans under natural selective pressures. Importantly, follow-up studies are needed to confirm that the identified genetic traits are responsible for altered host tropism.

Both types of comparative sequence approaches suffer from several significant limitations. First, the success of comparative sequence analysis is constrained by the quality and availability of existing genetic surveillance data. Relatively few sequences are available from relevant animal reservoirs, including bats and camels (for MERS). The only published camel sequences are from the Middle East, precluding the study of camel viruses from different geographic regions where spillover has/has not occurred. For the study of human epidemic CoVs, a limited number of SARS sequences are available from the 2002 – 2003 outbreak, and because MERS transmission chains have been relatively short, MERS data are of limited utility for studying adaptation mechanisms in humans. Of note, analysis of SARS epidemic strains reveals only one evolutionary pathway for adaptation to humans, which may represent one of several possible mechanisms. A second limitation is that, due to the large size of the CoV genome (27-32 kb) and the genetic diversity of coronaviruses in nature, there are a very large number of genetic differences between any two CoV strains, only a subset of which are likely to be important for cross-species adaptation.[1224] Because of that "noise," sequence comparisons are realistically limited to known regions of interest, precluding discovery of novel factors that are involved in host adaptation. Due to the fact that only a few proteins have been shown to be involved in cross-species adaptation and the function of most CoV proteins is unknown, this limited focus represents a critical shortcoming of the comparative sequence analysis approach. Although this limitation could be partially addressed by comparing sequences of paired animal and human isolates (e.g., MERS isolates from infected humans and the camels that are the likely sources of the infection), few such paired sequences are available. Third, this approach is reactive, limited to the study of mechanisms underlying adaptation of CoVs that have already evolved to broaden or alter their host tropism (e.g., SARS and MERS). The mechanisms driving adaptation of other CoVs to new hosts may be different. Of note, MERS does not efficiently infect and transmit in humans, unlike SARS, thus analysis of MERS sequences is limited to the discovery of traits that are associated with partial adaptation to humans. Finally, analysis of historical sequences cannot identify traits that were lost or negatively selected during adaptation (i.e., evolutionary pathways not taken) and thus provides a static view of evolutionary mechanisms underlying cross-species adaptation.

Conceptually similar to "Spike swapping" experiments, several alternative approaches seek to define the breadth of host tropism conferred by a given Spike protein. The first approach involves testing whether MERS- or SARS-CoVs can infect cells derived from various non-human host species such as bats or cells that do not naturally express CoV receptor proteins but have been engineered to ectopically express receptor proteins from various species. This approach cannot be used for most animal CoVs, which cannot be grown efficiently in cell culture to produce infectious material for laboratory assays. Alternatively, two virus-free approaches can provide information about compatible Spike-host interactions: (1) *in vitro* binding assays using recombinant Spike proteins and host receptor proteins from different species and (2) cell culture-based binding and virus entry assays using non-CoVs (e.g., murine leukemia virus) that are pseudotyped with CoV Spike proteins. These *in vitro* systems can also be used to confirm that amino acid substitutions in the Spike protein are necessary and sufficient to alter host receptor binding and cell entry capabilities.

The major limitation associated with these virus-free approaches is that results may not be recapitulated in the context of the wild type virus, as the virus context influences presentation of surface epitopes. CoV researchers reported cases of both false positive and false negative results when using pseudotyped viruses compared to wild type viruses.[1225] Additionally, results from either virus-free approach may not be conserved in a different strain context, and traits that promote binding of pseudotyped viruses to a particular cell type may not be critical for adaptation to human hosts. Finally, these approaches are

[1224] Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology* 84: 3134-3146.

[1225] (2015b) Interviews with coronavirus researchers.

currently used to investigate the role of the Spike-receptor interaction in host restriction only and are fundamentally limited to the investigation of known mammalian adaptation factors.

Structural modeling of Spike-receptor interactions, based on crystal structures of Spike-receptor complexes, can also be used to identify amino acid residues in the Spike protein that may be important determinants of host restriction. Though useful for generating hypotheses about mutations that may alter host tropism, all predictions must be experimentally confirmed.

In principle, Loss of Function (LoF) approaches could also be used to study mechanisms underlying cross-species adaptation, through the identification of genetic traits that are necessary for efficient infection of a particular host (i.e., screening mutants for reduced infectivity). However, because SARS, MERS, and bat CoVs do not naturally cause disease small laboratory animals, LoF approaches are not viable for the study of mechanisms underlying cross-species adaptation using wild type viruses. Notably, LoF approaches have been used to explore the genetic traits that are necessary for the mouse-adapted SARS strain to efficiently infect mice, by reverting adaptive mutations individually and in combination using site-directed mutagenesis and characterizing the infectivity of mutants.[1226] However, the mouse-adapted strain was originally generated using GoF approaches (i.e., serial passaging of SARS-CoV in mice). Although cell culture systems could, in principle, be used for LoF studies involving SARS and MERS, *in vitro* studies can provide minimal information about cross-species adaptation because the interaction of a virus with the host immune system is a critical facet of adapting to new hosts. No LoF studies using cell culture systems were identified in the scientific literature.

15.1.3.1.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The scientific knowledge benefits and limitations of all GoF and alt-GoF approaches discussed in this section, with respect to the ability of each approach to provide insight into the mechanisms underlying cross-species adaptation, are summarized in Table 15.1. Together, this analysis reveals that serial passaging, a GoF approach that alters host range, is **uniquely capable** of identifying *novel* viral genetic traits and factors that contribute to cross-species adaptation. Moreover, to elucidate the molecular mechanisms underlying the role of the Spike-receptor interaction in host adaptation, testing the phenotypic consequences of mutations in animal CoV Spike proteins in the context of a chimeric virus generated through GoF approaches provides a higher level of certainty in the validity of the results than similar confirmatory experiments using recombinant proteins or pseudotyped viruses. However, laboratory results in model systems may not translate to adaptation of viruses to humans in nature. Conversely, sequence comparisons, an alt-GoF approach, are uniquely capable of identifying genetic traits that are associated with mammalian adaptation across a variety of strains as well as discovering genetic markers that are definitively associated with human adaptation. However, the causality of markers identified through sequence analysis must be confirmed with a GoF experiment, and the utility of the comparative sequence approach is severely compromised by the poor state of genetic surveillance for CoVs in human and animal populations and the fact that it is limited to analysis of strains that have caused human infections.

[1226] Frieman M *et al* (2012) Molecular determinants of severe acute respiratory syndrome coronavirus pathogenesis and virulence in young and aged mouse models of human disease. *Journal of virology* 86: 884-897

Table 15.1. Comparison of GoF Approaches and Corresponding Alt-GoF Approaches That Benefit Scientific Knowledge: How Do Animal CoVs Adapt to Humans? What Are the Genetic and Phenotypic Traits Underlying Adaptation to Humans?

Experimental approach	Benefits	Limitations
GoF #1 [4a]: (*In vitro* approach) Serial passaging of virus in cells derived from non-natural host organism	• Identify **novel genetic traits** that are *sufficient* to alter host tropism, **for any virus** • Identify **novel viral factors** that are involved in cross-species adaptation, **for any virus**	• Associative – whether mutations are necessary for adaptation must be experimentally confirmed • Simplicity of model system – provides limited information about cross-species adaptation, and results may not be relevant for *in vivo* adaptation • Translatability – results from model systems may not translate to human infections • Narrow breadth – results may not generalize to other CoV strains
GoF #2 [4b]: (*In vivo* approach) Serial passaging of virus in non-natural host organism (e.g., mice)	• Identify **novel genetic traits** that are *sufficient* to alter host tropism, **for any virus** • Identify **novel viral factors** that are involved in cross-species adaptation, **for any virus** • Provides **in-depth information** about the evolutionary mechanisms underlying cross-species adaptation	• Associative – whether mutations are necessary for adaptation must be experimentally confirmed • Translatability – results from model systems may not translate to human infections • Artificiality – lab-directed evolution may not mimic natural selection • Narrow breadth – results may not generalize to other CoV strains
GoF #3 [5,6]: Targeted genetic modification to replace all or part of the CoV Spike protein with the Spike protein from another CoV species • Animal CoV + SARS Spike • SARS/MERS CoV + animal CoV Spike Characterize phenotypic properties of chimeric virus and compare sequences of animal CoV and SARS/MERS Spike proteins	• Define the breadth of host tropism conferred by a particular Spike protein • Identify amino acid substitutions within the Spike protein that may mediate host restriction • Gain insight into the potential for bat CoVs to adapt to humans	• Limited to studying the role of the Spike protein in cross-species adaptation • Chimeric viruses may behave differently than wild type viruses • Associative – whether substitutions are necessary and sufficient for host restriction must be experimentally confirmed

Table 15.1. Comparison of GoF Approaches and Corresponding Alt-GoF Approaches That Benefit Scientific Knowledge: How Do Animal CoVs Adapt to Humans? What Are the Genetic and Phenotypic Traits Underlying Adaptation to Humans?

Experimental approach	Benefits	Limitations
GoF #4 [7]: Targeted genetic modification of virus to introduce mutation(s) shown to be associated with adaptation to new hosts • Characterize ability of mutant virus to infect new cell type or animal	• Identify genetic traits that are **necessary and sufficient** to alter host tropism • **Confirm** viral factors that are involved in cross-species adaptation • Gain insight into mechanisms underlying virus adaptation to new hosts, including identification of underlying phenotypes	• Translatability – results from model systems may not translate to human infections • Narrow breadth – results may not generalize to other CoV strains
Alt-GoF #1 [4]: (virus free) Comparative analysis of surveillance data to identify genetic markers associated with adaptation to humans: • Animal versus human epidemic strains • Animal strains from different geographic regions where spillover of animal virus into human population has/has not occurred • Human epidemic CoV strains from different time points during an outbreak	• Identify genetic traits that are associated with **adaptation to humans** under **natural selective pressures** o Identify conserved traits, if large numbers of sequences are analyzed • Gain insight into the evolutionary mechanisms underlying cross-species adaptation	• Utility and success of approach is constrained by the quality and availability of genetic surveillance data • Bias – limited to investigation of known genetic regions of interest • Reactive – limited to the study of CoVs that have already caused human infections (i.e., SARS and MERS) • Associative – whether mutations are necessary and sufficient for adaptation must be experimentally confirmed • Static – evolutionary insight is limited because historical isolates represent discrete events along an evolutionary continuum

Table 15.1. Comparison of GoF Approaches and Corresponding Alt-GoF Approaches That Benefit Scientific Knowledge: How Do Animal CoVs Adapt to Humans? What Are the Genetic and Phenotypic Traits Underlying Adaptation to Humans?

Experimental approach	Benefits	Limitations
Alt-GoF #2 [6]: Test whether SARS or MERS can infect cells derived from non-human host species (e.g., bat, mouse, etc.) or can infect receptor-null human cells that are ectopically expressing receptor proteins • Test whether cells can be infected with animal-origin virus • Compare sequences of human and animal-origin host receptors to identify amino acids associated with host restriction	• Define the breadth of host tropism conferred by a particular Spike protein • Identify amino acid substitutions within the Spike protein that may mediate host restriction	• Approach cannot be used for animal CoVs that cannot be grown efficiently in cell culture • Associative – whether substitutions are necessary and sufficient for host restriction must be experimentally confirmed
Alt-GoF #3 [5,8]: • *In vitro*, virus free: o *In vitro* binding assays using recombinant CoV Spike proteins and host cell receptor proteins • In cells, pseudotyped virus: o Test host cell binding and entry using virus pseudotyped with CoV Spike proteins • Targeted genetic modification of Spike proteins to introduce mutations associated with altered host range in either context	• Define the breadth of host tropism conferred by a particular Spike protein • Identify amino acid substitutions that are necessary and sufficient to alter host tropism	• Simplicity of model system – results may not be recapitulated in the context of the wild type virus • Narrow breadth - results may not generalize to other CoV strains • Translatability – traits that promote binding to a particular cell type may not be critical for adaptation to human hosts • Limited to studying the role of the Spike protein in cross-species adaptation
Alt-GoF #4 [7]: (*in vitro*, virus free) Structural modeling of Spike-receptor interactions, based on crystal structures of Spike-receptor protein complexes	• Predict amino acid substitutions within the Spike protein that may mediate host restriction	• Predictive – phenotypic consequences of substitutions must be experimentally confirmed • Simplicity of model system – may not reflect virus-host cell interactions • Limited to studying the role of the Spike protein in cross-species adaptation

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental information).*

15.1.3.2 Scientific Knowledge Gap 2: How Do SARS and MERS Coronaviruses Cause Disease? What Are the Critical Viral Virulence Factors and Viral Genetic Determinants of Virulence?

Why SARS and MERS coronaviruses cause severe respiratory infections while other human coronaviruses cause mild to moderate illness is unknown.[1227] Specifically, the viral genetic and phenotypic traits underlying the enhanced pathogenicity of SARS and MERS relative to other human coronaviruses are poorly understood, and only a few viral virulence factors have been identified and characterized (such as the CoV Spike protein, which mediates viral entry into host cells). As there are no FDA-licensed vaccines or therapeutics for SARS or MERS, research in this area is important not only for increasing basic science knowledge about coronavirus biology but also for identifying potential new targets for therapeutics or for attenuation, for the purpose of developing live attenuated vaccines (LAVs). This benefit to MCM development will be discussed in more detail in Section 17.1.4, below.

15.1.3.2.1 Potential Benefits and Limitations of GoF Approaches

Serial passaging of CoVs in cell culture or laboratory animals, which selects for enhanced fitness (*in vitro*) or enhanced virulence (*in vivo*), is a GoF approach that enables the identification of mutations associated with enhanced fitness/virulence. Identification of virulence-associated mutations can lead to the discovery of new viral virulence factors and provides a foundation for follow-up studies investigating the mechanistic basis of the enhanced fitness/virulence phenotype observed in emergent viruses. As above, a key benefit of this approach is the ability to generate and identify novel mutations and viral proteins that contribute to fitness/virulence, without prior knowledge about viral virulence factors. Moreover, this approach can be performed with any coronavirus that is capable of infecting appropriate cell culture or animal model systems, including SARS-CoV, MERS-CoV, and chimeric animal-human CoVs used as tools for the study of animal CoVs that cannot be grown in model systems (discussed further below). *In vivo* serial passaging can provide a wider breadth of information than the *in vitro* approach because replicative fitness, though a component of virulence, does not necessarily correlate with virulence *in vivo*. For example, infected animals that are symptomatic and asymptomatic may exhibit similar viral loads, demonstrating that disease pathology is not simply caused by viral replication but also by the interaction of a virus with the host immune system. The roles of complex host-virus interactions can only be studied in the context of an animal model system (although underlying phenotypes can be studied *in vitro*). For both *in vitro* and *in vivo* model systems, insights may not translate to human infections, and viral factors and phenotypes that contribute to virulence in the CoV strain under study may not generalize to other CoV strains.

A second GoF approach for studying virulence involves targeted genetic modification of wild type viruses to introduce mutations that are associated with enhanced fitness/virulence, which demonstrates that such markers are *necessary* and *sufficient* to enhance fitness/virulence. Of note, these mutations can be discovered through a GoF approach, such as serial passaging, or an alt-GoF approach, such as comparative sequence analysis (discussed below). This information provides a strong foundation for follow-up studies investigating the mechanistic basis of the enhanced virulence phenotype, though mutations that are found to enhance virulence in model systems may not translate to increased virulence during human infections.

15.1.3.2.2 Potential Benefits and Limitations of Alt-GoF Approaches

Several alternative approaches can also be used to study pathogenicity. Two types of comparative sequence analysis can provide insight into viral genetic traits that may contribute to virulence. First,

[1227] (2015b) Interviews with coronavirus researchers.

comparative sequencing of SARS-CoV and MERS-CoV epidemic strains with varying levels of virulence can lead to the identification of mutations associated with enhanced virulence. A strength of this approach relative to serial passaging is that comparative sequence analysis uncovers genetic variation that is specially associated with enhanced virulence in humans.[1228,1229] However, this approach is limited to CoVs that have already produced epidemics in humans, i.e., SARS-CoV and MERS-CoV. The success of this approach depends on the availability of a wide breadth of surveillance data accompanied by epidemiological data about the clinical severity and case fatality rates of particular strains or groups or strains. In addition, the fact that SARS and MERS preferentially cause severe disease in patients who are elderly, immunocompromised, and/or who suffer from co-morbidities complicates the interpretation of genetic surveillance data. Because disease pathology can be exacerbated by host factors, such as age, as well as viral factors, high-quality "metadata" about relevant host factors (e.g., age, immune status, pre-existing medical conditions, etc.) is needed to control for host factors so that sequences can be appropriately "binned" into low- and high-virulence categories for comparison.[1230,1231,1232] While SARS-CoV strains from the early, middle, and late phases of the 2002 – 2003 epidemic have been found to exhibit varying levels of virulence (and have been used for comparative sequence analysis studies), genetic surveillance data for MERS are limited. Finally, given the large size of the CoV genome and genetic diversity among wild type CoV sequences, sequence comparisons are practically limited to pre-determined regions of interest, which precludes identification of novel virulence factors.

A second sequence-based approach involves analyzing the evolution of CoVs over time. Understanding which regions of the genome mutate and which do not can provide insight into which regions are likely to be critical for the virus life cycle. Although these regions/factors may not be involved in virulence per se, this approach may be useful for identifying promising therapeutic targets. However, the utility of this approach is also limited by the number of available CoV sequences.

Loss of Function (LoF) studies, which involve knocking out or otherwise hampering the function of a gene of interest (or its product) and screening for attenuated fitness (*in vitro*) or virulence (*in vivo*), represent another alternative approach for the discovery of viral virulence factors and genetic traits associated with virulence. Though this approach enables the identification of novel virus proteins that are necessary for enhanced fitness/virulence, the simple discovery of a novel virulence factor does not provide information about its potential function. Conversely, a random mutagenesis approach may provide insight into the mechanistic basis of virulence but is highly inefficient because of the number of potential targets in the CoV genome. The major drawback of LoF screens is that losing the functionality of a virus protein, either through gene knockout of mutagenesis, may indirectly attenuate virulence, so that gaining meaningful information about virulence mechanisms may be difficult using this approach. One strategy for identifying potentially interesting gene targets for LoF studies is to examine CoV sequences for the presence of conserved enzymatic motifs. However, a limited number of CoV enzymes contain recognizable motifs (e.g., the RNA-dependent RNA polymerase), and CoV accessory proteins are distinctive among CoVs and distinctive in nature.[1233] Thus, a LoF approach that relies on targeted mutagenesis is primarily limited to the investigation of virulence-enhancing mutations in known virulence

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[1228] Qu XX *et al* (2005) Identification of two critical amino acid residues of the severe acute respiratory syndrome coronavirus spike protein for its variation in zoonotic tropism transition via a double substitution strategy. *J Biol Chem* 280: 29588-29595

[1229] Chinese SMEC (2004) Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science* 303: 1666-1669

[1230] Roberts A *et al* (2007) A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS pathogens* 3: e5

[1231] Peiris JS *et al* (2003) Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 361: 1767-1772

[1232] Assiri A *et al* (2013) Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *The Lancet Infectious diseases* 13: 752-761

[1233] (2015b) Interviews with coronavirus researchers.

factors. For both LoF strategies, a limited number of mutants can be screened for attenuated virulence *in vivo*, due to the labor, expense, and ethical considerations associated with the conduct of animal experiments. Though high-throughput screening for reduced replicative fitness can be conducted using cell culture systems, as discussed above, replicative fitness does not necessarily correlate with virulence and represents only one of the phenotypes underlying virulence. Thus, *in vitro* LoF screening approaches may lead to false negative and false positive results and can only target a fraction of the virulence factor space. Finally, it is noted that knocking out the function of an unknown viral protein can lead to a loss or gain of virulence, depending on the function of the protein. Notably, even genetic manipulations that are predicted to attenuate virulence based on preliminary *in vitro* work can lead to enhanced virulence when tested in an *in vivo* model system.[1234]

LoF approaches can also be used to confirm that a particular trait is *necessary* for enhanced virulence. However, because virulence is a complex, multi-genic trait, knocking out the function of one gene or introducing a mutation into one gene may be sufficient to attenuate virulence but provides an incomplete picture of the role of that particular protein. As above, mutations that are found to enhance virulence in model systems may not translate to increased virulence during human infections.

15.1.3.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The scientific knowledge benefits and limitations of all GoF and alt-GoF approaches discussed in this section, with respect to the ability of each approach to provide insight into the mechanisms underlying CoV virulence, are summarized in Table 15.2. Taken together, serial passaging for the selection of CoV strains with enhanced pathogenicity in animals or fitness in cell culture, a GoF approach, is the most efficient and effective method for identifying novel genetic traits and/or viral factors that contribute to virulence in any coronavirus strain. However, results in cell culture or animal model systems may not translate to human disease. The alternate approaches have several drawbacks. While screening gene knockout viruses *in vitro* represents a viable approach for the discovery of novel virulence factors, this LoF approach is limited to the identification of proteins that influence replicative fitness, only one component of virulence, and may uncover factors that attenuate virulence for trivial reasons. The main drawback of both the GoF and LoF approaches is that insights gleaned from model systems may not translate to human infection. To that end, comparatively analyzing the sequences of SARS/MERS strains with varied levels of virulence can provide direct insight into genetic traits that are associated with pathogenicity in humans. However, this approach is limited to the study of SARS and MERS and is significantly constrained by shortcomings in the quality and availability of existing genetic surveillance data. In addition, any hypothesis generated through comparative sequence analysis must be experimentally confirmed. The phenotypic consequences of mutations that are associated with enhanced virulence can be validated using GoF approaches, which are uniquely capable of demonstrating that mutations are necessary and sufficient to enhance virulence, or LoF approaches, which can demonstrate that mutations are necessary for enhanced virulence only. Complex, multi-genic traits such as virulence are difficult to tease apart using solely LoF approaches because LoF provides limited information about how proteins cooperate to enhance virulence. However, because the value of the information gleaned from both LoF and GoF approaches depends on the relevance of artificially manipulated viruses to nature, using both approaches to confirm the role of a particular mutation or phenotype strengthens any conclusion.

[1234] Eckerle LD *et al* (2007) High fidelity of murine hepatitis virus replication is decreased in nsp14 exoribonuclease mutants. *Journal of virology* 81: 12135-12144

Table 15.2. Comparison of GoF Approaches and Corresponding Alt-GoF Approaches That Benefit Scientific Knowledge: How Do CoVs Cause Disease? What Are the Critical Viral Virulence Factors and Viral Genetic Determinants of Virulence?

Experimental approach	Benefits	Limitations
GoF #1 [2]: (*In vitro* approach) Serial passaging of virus in cells	• Identify **novel genetic traits** that are *sufficient* to enhance fitness in cell culture, **for any virus** • Identify **novel viral factors** that may contribute to virulence, **for any virus**	• Associative – whether mutations are necessary to enhance fitness must be experimentally confirmed • Simplicity of model system – replicative fitness is one component of virulence and does not necessarily correlate with virulence *in vivo* • Translatability – results from model systems may not translate to human infections • Narrow breadth – results may not generalize to other CoV strains
GoF #2 [2]: (*In vivo* approach) Serial passaging of virus in animals	• Identify **novel genetic traits** that are *sufficient* to enhance virulence, **for any virus** • Identify **novel viral factors** that may contribute to virulence, **for any virus**	• Associative – whether mutations are necessary to enhance virulence must be experimentally confirmed • Translatability – results from model systems may not translate to human infections • Narrow breadth – results may not generalize to other CoV strains
GoF #3 [3]: Targeted genetic modification of virus to introduce mutation(s) shown to be associated with enhanced fitness/virulence • Characterize fitness/virulence of mutant in cell culture or animal model systems	• Identify genetic traits that are **necessary and sufficient** to enhance fitness/virulence • **Confirm** viral factors that contribute to virulence • Gain insight into mechanisms underlying pathogenesis, including identification of underlying phenotypes	• Translatability – results from model systems may not translate to human infections • Narrow breadth – results may not generalize to other CoV strains

Table 15.2. Comparison of GoF Approaches and Corresponding Alt-GoF Approaches That Benefit Scientific Knowledge: How Do CoVs Cause Disease? What Are the Critical Viral Virulence Factors and Viral Genetic Determinants of Virulence?

Experimental approach	Benefits	Limitations
Alt-GoF #1 [1]: (virus free) Comparative sequence analysis of human epidemic CoV strains with varying levels of virulence	• Identify genetic traits that are associated with enhanced virulence **in humans** • Identify conserved traits, if large numbers of sequences are analyzed	• Utility and success of approach is constrained by the quality and availability of genetic surveillance data • Host factors such as age complicate interpretation of virulence data • Bias – limited to investigation of known genetic regions of interest • Reactive – limited to the study of CoVs that have already caused human infections (i.e., SARS and MERS) • Associative – whether mutations are necessary and sufficient for adaptation must be experimentally confirmed
Alt-GoF #2 [2]: (virus free) Comparative analysis of CoV sequences over time, to which regions of the genome mutate	• Identify genetic regions that may be critical for the virus life cycle	• Predictive – whether regions contribute to virulence must be experimentally confirmed • Utility and success of approach is constrained by the quality and availability of genetic surveillance data

Table 15.2. Comparison of GoF Approaches and Corresponding Alt-GoF Approaches That Benefit Scientific Knowledge: How Do CoVs Cause Disease? What Are the Critical Viral Virulence Factors and Viral Genetic Determinants of Virulence?

Experimental approach	Benefits	Limitations
Alt-GoF #3 [3]: (Loss of Function) Forward genetic screen to identify mutations expected to attenuate replication *in vitro* or virulence *in vivo* • Random mutagenesis of known virulence factors to generate libraries of mutant viruses, followed by screening of mutants for attenuated replication/virulence • Knock out function of individual genes and screen for attenuated replication/virulence	• Identify genetic traits or viral factors that are **necessary** for enhanced virulence	• Triviality – losing the function of a virus protein may indirectly attenuate virulence • Bias - Targeted mutagenesis strategies primarily limited to the investigation of known virulence factors • Inefficient – limited number of mutants can be screened *in vivo* • Simplicity of *in vitro* model system – replicative fitness does not necessarily correlate with virulence • Unpredictable - knocking out the function of a protein can lead to a gain or loss of virulence • Mutations that are necessary for enhanced virulence may not be sufficient to enhance virulence in a different genetic context

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental Information).*

15.1.4 Benefits of GoF-Derived Model Systems

Model systems that can be efficiently infected by CoVs, support robust viral replication, and mimic human disease pathogenesis are essential for the experimental study of CoV biology and for the development of MCMs. GoF approaches that expand the host range of CoVs are used for the development of *in vitro* and *in vivo* model systems for SARS, MERS, and animal-origin CoVs (e.g., SARS/MERS-like bat CoVs, civet CoVs, etc.)

15.1.4.1 GoF Benefits to the Development of in Vitro Model Systems

Cell culture systems that can be infected and support robust replication of CoVs are essential for the generation of viral stocks that are used for *in vitro* and *in vivo* experiments and for investigating basic mechanisms of CoV infection using cell biological methods. Both SARS and MERS readily and persistently infect human cell lines, but many animal CoVs cannot be cultured *in vitro*, including SARS/MERS-like bat CoVs and zoonotic SARS strains from civets.[1235,1236] Specifically, many animal CoVs do not naturally infect human cell lines, and some bat CoVs cannot be isolated in bat cell lines either. Even for those bat CoVs that are capable of naturally infecting bat cells, adaptation to standard mammalian cell culture systems is desirable because bat cells are more difficult to culture and to manipulate experimentally (e.g., transfect, etc.) than human cell systems.[1237,1238] Therefore, new *in vitro* model systems for animal CoVs are needed in order to effectively study the properties of these SARS/MERS progenitor viruses and to assess their potential to adapt to humans.

15.1.4.1.1 In Vitro Model Systems Developed Using GoF Approaches

Two GoF approaches can be used to adapt animal CoVs for growth in human cells: serial passaging in cell culture and "Spike swapping." First, serial passaging in cell culture selects for viruses that are better able to bind, infect, and replicate within human cells. This approach may not be successful if the initial capacity of the virus to infect human cells is very low. For example, Becker and colleagues were unable to recover and passage a consensus bat SARS-like CoV (Bat-SCoV, constructed from four bat SARS-like CoV sequences) in human cells.[1239] The main limitation of this approach is that serial passaging may lead to the acquisition of mutations that alter the biological behavior of the virus in unexpected ways, which may limit the relevance of any results to the wild type virus.

A second approach involves "Spike swapping," targeted genetic modification to replace all or part of an animal CoV Spike protein with the SARS Spike protein to generate a recombinant chimeric virus (i.e., animal CoV + SARS Spike). Because the Spike protein is a major determinant of host tropism, this replacement often enables the chimeric animal-SARS virus to infect and replicate within human

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[1235] Sheahan T *et al* (2008) Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. Ibid. 82: 2274-2285

[1236] Agnihothram S *et al* (2014) A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *mBio* 5: e00047-00014

[1237] Huynh J *et al* (2012) Evidence supporting a zoonotic origin of human coronavirus strain NL63. *Journal of virology* 86: 12816-12825

[1238] Yang Y *et al* (2015) Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. Ibid. 89: 9119-9123

[1239] Becker MM *et al* (2008) Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. *Proceedings of the National Academy of Sciences of the United States of America* 105: 19944-19949

cells.[1240,1241] Of note, chimeric viruses can also serve as a starting point for the development of a mouse-adapted strain, discussed in more detail in the subsequent section.[1242,1243,1244] One benefit of this approach is that, because the SARS Spike protein mediates entry into a variety of cell types, chimeric viruses can likely be used to infect both immortalized and primary cell lines (such as human airway epithelial cells, the site of primary infection of coronaviruses and as such a more relevant model system for the study of CoV infection than immortalized cell lines).[1245] The main drawbacks of this approach are that the behavior of the chimeric virus may not reflect that of the wild type virus and that chimeric viruses cannot be used to study the function of the animal Spike protein.

15.1.4.1.2 Alternative in Vitro Model Systems That Do Not Involve the Use of GoF Approaches

Several alternative model systems, which do not involve GoF approaches, may permit the study of animal CoVs in cell culture: use of cell lines derived from the natural host (e.g., bat), use of cell lines that are naturally permissive to infection, and the development of human cell lines that are sensitized to infection with animal CoVs. First, some bat CoVs are naturally capable of replicating within bat cell lines, such as a bat SARS-like CoV isolated in 2013 which is thought to be a progenitor strain for SARS.[1246] However, there are several limitations associated with the use of bat cell lines. Bat cell lines are much less experimentally tractable than human cell lines, as fewer reagents are available and the cells are more difficult to transfect than human cells.[1247,1248] Also, some bat CoVs do not infect existing immortalized bat cell lines (only a few are available), which restricts their utility as a model system for emerging CoVs.[1249,1250]

A second alternative involves the use of naturally permissive cell lines. For example, the SARS-like bat CoV strain described above was found to naturally replicate in Vero cells (derived from African green monkeys), human alveolar basal epithelial cells and pig kidney cells.[1251] Interestingly, this strain replicated to higher titers in Vero cells than in bat kidney cells, demonstrating that cells derived from a natural host species do not necessarily represent a superior model system than cells derived from a non-natural host species. However, many bat CoVs, such as the MERS-like virus HKU5, cannot be cultured in standard *in vitro* systems, limiting the utility of this approach.[1252] In addition, CoVs that are found to

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1240 For example, swapping the Spike ectodomain from SARS into the backbone of Bat-SCoV permitted replication of the chimeric virus in a variety of human cell types.

1241 Becker MM *et al* (2008) Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. *Proceedings of the National Academy of Sciences of the United States of America* 105: 19944-19949

1242 In this case, the Spike protein from the mouse-adapted SARS strain (which contains one amino acid substitution relative to the WT SARS protein) is used to generate the chimeric virus.

1243 Becker MM *et al* (2008) Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. *Proceedings of the National Academy of Sciences of the United States of America* 105: 19944-19949

1244 Agnihothram S *et al* (2014) A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *mBio* 5: e00047-00014

1245 Dijkman R *et al* (2013) Isolation and characterization of current human coronavirus strains in primary human epithelial cell cultures reveal differences in target cell tropism. *Journal of virology* 87: 6081-6090

1246 Ge XY *et al* (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538

1247 Yang Y *et al* (2015) Two Mutations Were Critical for Bat-to-Human Transmission of Middle East Respiratory Syndrome Coronavirus. *Journal of virology* 89: 9119-9123

1248 Huynh J *et al* (2012) Evidence supporting a zoonotic origin of human coronavirus strain NL63. Ibid. 86: 12816-12825

1249 ibid.

1250 Agnihothram S *et al* (2014) A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *mBio* 5: e00047-00014

1251 Ge XY *et al* (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538

1252 Agnihothram S *et al* (2014) A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *mBio* 5: e00047-00014

naturally infect and replicate within bat cells or human cells may yield progeny virus incapable of further infection.[1253]

A final alternative involves sensitizing host cells to infection through ectopic expression of the receptor protein from the natural host species (or another permissive host species). For example, Ge and colleagues demonstrated that the bat SARS-like CoV described above is capable of infecting and replicating within HeLa cells expressing the ACE2 receptor from civets or bats, demonstrating the potential utility of this strategy for development of an *in vitro* model system for the study of bat CoVs.[1254] As this system does not account for host factors governing viral entry and replication other than the host receptor, whether this strategy will permit replication of a broad range of emerging CoVs is unknown. Additionally, this strategy cannot be used for primary cell lines, which are not readily transfectable, and overexpression of the receptor may alter the process of infection, leading to artefactual results. Finally, within each alternative system, wild type viruses may not replicate to high enough titers for experimental use without serial passaging to select for higher-yield viruses.

15.1.4.1.3 Summary – Benefits of GoF-Derived in Vitro Model Systems Relative to Alternative Model Systems

The strengths and limitations of each *in vitro* model system analyzed in this section are summarized in Table 15.3. Studying SARS/MERS-like animal CoVs, thought to be precursors for SARS/MERS or to have similar potential to spill over into human populations, provides important insight into how SARS and MERS emerged from their animal reservoirs to infect humans. In addition, defining which animal CoVs have potential to adapt to humans can guide efforts to develop broad-spectrum MCMs for emerging CoVs. However, most animal CoVs grow poorly, if at all, in standard cell culture systems. GoF approaches have **unique potential** to enable the development of *in vitro* model systems for the study of any animal CoV in a variety of cell types, including immortalized cell lines and relevant primary cell lines such as human epithelial airway cells. Alternatives to GoF have significant shortcomings. Only a subset of animal CoVs identified to date can be cultured in bat, human, or other standard cell lines, limiting the utility of using naturally permissive cell lines for *in vitro* studies. While ectopic expression of permissive receptor proteins in a common cell line has been shown to permit replication of several CoVs, this strategy is limited to cell lines that can be readily transfected (i.e., not primary cell lines) and overexpression of the host receptor may alter the biology of infection, limiting the relevance of results from this system.

[1253] Sheahan T *et al* (2008) Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. *Journal of virology* 82: 2274-2285

[1254] Ge XY *et al* (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538

Table 15.3. CoVs: Summary of the Benefits of GoF Approaches That Inform the Development of *in vitro* Model Systems

Scientific Knowledge and MCM Development Benefits – Development of *in Vitro* Model Systems for SARS-CoV and MERS-CoV

Model system	Benefits	Limitations
GoF: Animal CoV adapted for growth in human cell lines • Serial passage of animal CoVs in human cell lines [1, 4a] • Targeted genetic modification to generate chimeric virus: animal CoV plus SARS Spike [5]	• Can be applied to any animal CoV • Adapted viruses can be used to infect a variety of cell types, including immortalized and primary cells • A wide variety of methods and reagents are available for human cell lines	• The behavior of adapted viruses may not reflect that of wild type viruses • Chimeric viruses cannot be used to study the function of the animal CoV Spike protein
Alt-GoF #1: Use of cell lines derived from the natural host (e.g., bat)	• Enables the use of wild type bat CoVs	• Few bat CoVs infect existing immortalized bat cell lines (few cell lines are available) ○ Progeny may be incapable of further infecting cells • Bat cell lines are less experimentally tractable than human cell lines
Alt-GoF #2: Use of naturally permissive cell lines	• Enables the use of wild type animal CoVs • Wild type viruses may replicate to higher titers than in cells derived from natural host	• Few bat CoVs can be cultured in standard *in vitro* systems ○ Progeny may be incapable of further infecting cells
Alt-GoF #3: Use of human cells that have been sensitized to infection • Ectopic expression of virus receptor from the natural host species	• Enables the use of wild type animal CoVs • A wide variety of methods and reagents are available for human cell lines	• Additional host factors play a role in virus entry and replication ○ Strategy may not be successful for all animal CoVs • Overexpression of the receptor may alter infection processes • Limited to the use of host cell lines that can be readily transfected

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental Information).*

15.1.4.2 GoF Benefits to the Development of Animal Model Systems

Animal models are essential for understanding the pathology of viral disease and for developing vaccines and therapeutics. Replication models are animals that support viral replication but do not mimic human disease, while pathogenesis models are those that support viral replication and emulate human pathologies. If suitable laboratory animals are not naturally susceptible to infection, animal models can be developed by adapting a wild type virus to the host through passaging or by adapting the host to the virus by transgenic expression of host viral receptors or other restriction factors. Adapted strains, transgenic animals, and naturally susceptible species have all been used to study SARS-CoV and MERS-CoV.

Appropriate animal models are critical for the development of new vaccines and therapeutics. To study vaccine efficacy, the model must show the ability of the vaccine to prevent pathology associated with infection following a challenge.[1255] In addition, under the FDA's Animal Efficacy Rule, vaccines and therapeutics against rare, emerging, or virulent agents such as SARS-CoV can achieve regulatory approval provided efficacy is demonstrated in multiple animal models that display clinical illness representative of human disease.[1256,1257] (Whether the Animal Rule applies to the development of MCMs targeting MERS-CoVs is uncertain, as the number and distribution of MERS cases in the Kingdom of Saudi Arabia may enable the conduct of clinical trials, which is preferable. This issue will be addressed on a case-by-case basis if sponsors seek approval of a MERS-CoV vaccine or therapeutic under the Animal Rule.)[1258] In the event that a sponsor seeks approval of a SARS-CoV vaccine or therapeutic under the Animal Rule, the sponsor must provide scientific justification that the animal used to study countermeasures exhibits key characteristics of human disease when exposed to the challenge agent and accurately predicts human responses. In sum, development of a pathogenesis model that adequately mirrors the route of infection, severity, clinical signs, and levels of mortality and morbidity seen in humans is critical for advancing countermeasure development and for satisfying the FDA Animal Rule.

15.1.4.2.1 Animal Model Systems Developed Using GoF Approaches

Virus Strains That Have Been Adapted to Laboratory Animals

Adaptation of a virus to a mammalian host through serial passaging is a commonly used method for creating pathogenesis models. Because this method results in an additional capability for the virus to infect and cause disease in a new host species (i.e., altered host range and enhanced pathogenicity in appropriate animal model systems), this method represents a GoF approach. As neither SARS-CoV nor MERS-CoV are capable of productively infecting mice to recapitulate human disease pathogenesis, mouse-adapted strains of SARS-CoV are preferred relative to use of the WT strain, and efforts to development a mouse-adapted MERS-CoV strain are ongoing.[1259] Mouse-adapted strains are important tools for the study of viral pathogenesis and of host factors involved in responses to infection. Use of mouse-adapted strains allows researchers to capitalize on the diversity of mouse-specific tools developed for the study of host immune responses, including many strains of knockout mice and reagents for manipulating host immune factors (e.g., antibodies for depletion of particular types of host immune cells). Lessons learned using mouse-adapted strains are likely to be applicable to humans because pathogenesis

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[1255] (2015b) Interviews with coronavirus researchers.
[1256] Sutton TC, Subbarao K (2015) Development of animal models against emerging coronaviruses: From SARS to MERS coronavirus. *Virology* 479-480C: 247-258
[1257] FDA. Product Development Under the Animal Rule: Guidance for Industry. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm399217.pdf. Last Update October 2015. Accessed November 23, 2015.
[1258] (2015m) Personal communication from FDA representative.
[1259] (2015b) Interviews with coronavirus researchers.

mechanisms, specifically virus entry mechanisms, are similar to those in humans.[1260] However, there is the possibility that unforeseen changes will arise during passaging that may affect pathogenesis and potentially complicate comparisons between mice and humans.[1261] Understanding how adaptation mechanisms alter the phenotypes under study is critical for the correct interpretation of results.[1262] In addition, adapted strains can be used to test whether candidate MCMs can prevent or reduce the pathology associated with human disease, which is important for advancing countermeasure development. The adapted strains of SARS-CoV have been used in vaccine development, representing a significant advance towards satisfying the FDA Animal Rule.[1263]

15.1.4.2.2 Alternative Animal Model Systems That Do Not Involve the Use of GoF Approaches

Transgenic Laboratory Animals That Have Been Sensitized to Infection

Use of transgenic animals expressing the human virus receptor is an alternative to the use of adapted viruses for hosts that are not permissive or do not recapitulate human disease pathology. Transgenic approaches have been used to develop mouse models for SARS-CoV and MERS-CoV. Transgenic models allow the direct study of wild type viruses, thus avoiding the concern that adaptive changes during passaging alter mechanisms of viral pathogenesis. Transgenic mice are important in countermeasure development because they can be used to establish that a therapy knocks down virus titers in a system with human receptors.[1264] For MERS-CoV, a transgenic approach can be used as a starting point for the creation of an adapted strain because mice do not naturally express the appropriate viral entry receptors.[1265] A variety of approaches have been used to create transgenic mouse models for SARS-CoV and MERS-CoV infection, but each technique results in a slightly different gene expression pattern and reproduces human disease symptoms to a different degree. As a result, the relevance of results about pathogenesis mechanisms and MCM efficacy is subject to significant caveats. Notably, to date, no animal model that includes a genetically modified host has been used to approve an FDA-regulated countermeasure under the Animal Rule.[1266]

Naturally Susceptible Species

Another alternative to the use of viruses that have been adapted to laboratory animals is the use of naturally susceptible hosts. However, laboratory animals that are naturally susceptible to infection with SARS-CoV and MERS-CoV have been found to support viral replication but remain asymptomatic or develop symptoms dissimilar to those in humans. SARS-CoV is capable of productively infecting mice, hamsters, ferrets, and several species of non-human primate, though not all species exhibit clinical signs or mortality. MERS-CoV, which utilizes a different entry receptor than SARS-CoV, exhibits a greater degree of host species restriction; replication is limited to some species of non-human primate and no small mammals are permissive to infection. Thus, for both SARS-CoV and MERS-CoV, naturally susceptible hosts function as replication models, not pathogenesis models.[1267]

1260 Ibid.
1261 Frieman, M., et al. (2012). "Molecular determinants of severe acute respiratory syndrome coronavirus pathogenesis and virulence in young and aged mouse models of human disease." J Virol **86**(2): 884-897.
1262 (2015b) Interviews with coronavirus researchers.
1263 Sutton TC, Subbarao K (2015) Development of animal models against emerging coronaviruses: From SARS to MERS coronavirus. *Virology* 479-480C: 247-258.
1264 (2015b) Interviews with coronavirus researchers.
1265 Ibid.
1266 (2015m) Personal communication from FDA representative.
1267 (2015b) Interviews with coronavirus researchers.

Replication models are used in vaccine and antiviral drug development to demonstrate diminished replication, an important proof of concept for medical countermeasures.[1268] Additionally, identifying a natural replication model is often the first step in creating a pathogenesis model. However, animals that do not recapitulate human disease pathogenesis have limited utility for investigating how viruses interact with host systems to cause disease, and asymptomatic replication models do not provide insights into pathogenesis or disease progression. Replication models also have limited utility for advanced MCM development. Replication models may provide easy metrics to demonstrate vaccine or drug efficacy (i.e., reduction in viral replication), but their lack of relevant symptomology could lead to the development and release of subpar or dangerous countermeasures.[1269] Specifically, therapeutics may cause unintended side effects or deleterious interactions with the host immune system, which are unpredictable and may not be observed in asymptomatic animal models.[1270] This concern is supported by the example of a SARS-CoV vaccine candidate, which was efficacious in non-human primate replication models but produced severe adverse side effects when tested in mouse pathogenesis models. After vaccinated mice were challenged with live SARS-CoV virus, the mice displayed an immunopathologic Th2-type response, which is predictive of a harmful response to the vaccine in humans.[1271] As a result, this vaccine candidate did not undergo clinical trials.

Alternative Coronaviruses That Are Naturally Pathogenic to Laboratory Animals - Mouse Hepatitis Virus

The coronavirus mouse hepatitis virus (MHV) has been used as a model to generate basic knowledge about coronavirus biology but cannot serve as a substitute for MERS-CoV or SARS-CoV for pathogenesis studies or MCM development studies. Adult mouse infections of MHV are usually asymptomatic. While infant mice exhibit pathology during infection, the symptoms and disease course do not mimic those of MERS-CoV or SARS-CoV. MHV has been useful for the study of mechanisms universal to coronaviruses, which has led to the discovery of generalizable information about coronavirus polymerases, proteases, and other nonstructural proteins.[1272] However, coronaviruses do not share the core machinery often targeted by antivirals or vaccines, and studies have shown that inhibitors that successfully target one coronavirus do not work for the other.[1273,1274] Thus, the efficacy of all countermeasures tested in the context of MHV infection must be confirmed using SARS-CoV or MERS-CoV. In addition, due to the unique features of SARS-CoV and MERS-CoV, pathogenesis, transmissibility, and the effects of SARS-CoV and MERS-CoV in humans cannot be studied using MHV.[1275]

Human Autopsy Data

Human autopsy data can be an alternative source of pathogenesis information. SARS associated lung pathology was described from examination of post-mortem tissue samples; however, pathologic changes associated with MERS have not been reported due to a lack of autopsy data.[1276] Autopsies are not often performed in Middle Eastern cultures, and data has not yet been shared from the most recent outbreak in

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1268 Ibid.
1269 Ibid.
1270 Ibid.
1271 Tseng, C. T., et al. (2012). "Immunization with SARS-CoV coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS-CoV virus." PLoS One **7**(4): e35421.
1272 (2015b) Interviews with coronavirus researchers.
1273 Ibid.
1274 Hilgenfeld R (2014) From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. *The FEBS journal* 281: 4085-4096
1275 (2015b) Interviews with coronavirus researchers.
1276 Gretebeck LM, Subbarao K (2015) Animal models for SARS and MERS coronaviruses. *Current opinion in virology* 13: 123-129.

the Republic of Korea.[1277] Human autopsy data is inherently correlative and is devoid of time series information, obscuring the order in which pathogenic effects occurred. Diversity in genetic backgrounds, life histories, and chronic conditions must all be taken into account and can complicate the identification of pathology caused by viral infection versus comorbidities. MERS-CoV has increased mortality rates in the elderly and those with pre-existing health conditions, so information from these individuals may not fully represent pathology seen in younger, otherwise healthy persons.

15.1.4.2.3 Summary – Benefits of GoF-Derived in Vitro Model Systems Relative to Alternative Model Systems

Model systems are essential for understanding the pathology of viral disease and for developing vaccines and therapeutics. The strengths and limitations of each *in vivo* model system analyzed in this section are summarized in Table 15.4. Mouse-adapted strains of SARS, which exhibit altered host range and enhanced virulence in mice relative to the wild type SARS virus, represent the only model system that recapitulates disease pathogenesis observed during human infections of SARS-CoV. As existing animal models for MERS-CoV do not replicate human disease pathology, mouse-adapted strains of MERS-CoV are expected to serve as the sole pathogenesis model for the study of MERS-CoV infection as well. As such, animal-adapted strains can be used to study many facets of disease pathogenesis, including the course of disease, the role of viral and host immune factors in disease pathology, and the role tissue tropism in disease pathology. Alternative model systems have critical drawbacks for the study of disease pathogenesis. Transgenic animals do not recapitulate the features of human disease because the engineered animals do not exhibit native expression patterns of viral receptor proteins. As a result, lessons learned about pathogenesis may not translate to humans, and transgenic animals cannot be used to study the role of tissue tropism in disease pathology. Most naturally susceptible hosts are asymptomatic or display dissimilar symptoms to humans and thus cannot be used to study disease pathogenesis. While human autopsy data are uniquely capable of providing insight into human disease pathology, limited autopsy data are available, and the static nature of the data and the presence of co-morbidities in many SARS/MERS patients complicate interpretation of the data.

The use of animal-adapted strains of CoVs is critical for advanced MCM development as well and provides significant advantages over the use of alternative model systems. Though transgenic animals and naturally susceptible hosts can be used to demonstrate that MCMs diminish viral replication, an important proof of concept for early stage MCMs, animal-adapted strains that replicate human disease pathology provide a much more robust system for demonstrating the safety and efficacy of MCM candidates. In addition, because adapted strains provoke a response from the host immune system, use of these strains can reveal MCM side effects or adverse reactions that are not seen in asymptomatic models.

[1277] (2015b) Interviews with coronavirus researchers.

Table 15.4. CoVs: Summary of the Benefits of GoF Approaches that Alter Host Tropism and Enhance Virulence		
Scientific Knowledge and MCM Development Benefits – Development of *in Vivo* Model Systems for SARS-CoV and MERS-CoV		
Model system	**Benefits**	**Limitations**
GoF [4b]: Animal-adapted SARS-CoV/MERS-CoV • Serial passage of SARS/MERS virus in animals (e.g., mice)	• Animal-adapted strains recapitulate the pathology of human disease o Suitable for the study of disease pathogenesis mechanisms o Robust system for testing the safety and efficacy of MCMs	• Mutations that arise during passaging may alter pathogenesis in unexpected ways, complicating comparisons between mice and humans
Alt-GoF #1: Use of naturally susceptible laboratory animal hosts	• Enables the use of wild type virus strains • Can be used to demonstrate that MCMs diminish viral replication	• Naturally susceptible hosts are asymptomatic or display different symptoms than humans o Cannot be used for the study of pathogenesis mechanisms o Weak system for testing the efficacy of MCMs o Do not display adverse reactions/side effects of MCMs
Alt-GoF #2: Use of transgenic animals: sensitize non-permissive host to infection through expression of virus entry receptor • Stable expression of human receptor (e.g., knock-in mouse) using universal or host promoter • Transient expression of human receptor (e.g., adenovirus vector-based transduction)	• Enables the use of wild type virus strains • Can be used to demonstrate that MCMs diminish viral replication in a system with human virus receptors	• Transgenic animals not mimic human pathogenesis due to different transgene expression patterns than in humans o Cannot be used to investigate tissue tropism, and pathogenesis mechanisms may not translate to humans o MCM testing results may not translate to humans
Alt-GoF #3: Use of human autopsy data from MERS-CoV cases	• Provides direct information about human pathology	• Data limited by infrequency of autopsies in Middle East • Mortalities are not representative of all cases o Higher incidence of mortality in patients with co-morbidities

Table 15.4. CoVs: Summary of the Benefits of GoF Approaches that Alter Host Tropism and Enhance Virulence		
Scientific Knowledge and MCM Development Benefits – Development of *in Vivo* Model Systems for SARS-CoV and MERS-CoV		
Model system	**Benefits**	**Limitations**
Alt-GoF #4: Use of alternative coronavirus: Mouse Hepatitis Virus (MHV)	• Can be used to gain insight into basic aspects of coronavirus biology	• Does not replicate human disease pathogenesis • Does not share core machinery often targeted by MCMs with SARS or MERS
** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental Information).*		

15.1.5 Benefits of GoF to Public Health/Medicine

GoF approaches have potential to benefit the development of vaccines and therapeutics for coronaviruses in two ways. First, scientific information gleaned using GoF approaches may inform the development of new medical countermeasures and supports their licensure. Second, model systems developed using GoF approaches can be used to demonstrate the safety and efficacy of candidate vaccines and therapeutics. This section evaluates the benefits of both types of GoF approaches for the development of vaccines and therapeutics, relative to alternative experimental approaches as well as alternative scientific and technical innovations that have the potential to similarly benefit MCM development.

For both vaccines and therapeutics, several different types of GoF research (i.e., different GoF phenotypes) can inform different stages of the MCM development and licensure process. To promote an understanding of the criticality of GoF approaches for the creation of new vaccines and therapeutics, it is necessary to first evaluate all GoF approaches that contribute to the vaccine development process (which includes multiple GoF phenotypes), and then evaluate all GoF approaches that contribute to the development of new therapeutics (which includes multiple GoF phenotypes). Within the vaccine and therapeutic sub-sections, the process of developing a vaccine or therapeutic, from development to licensure, is outlined and the role of GoF versus alternative approaches at each stage of the process is evaluated. (Note that this structure is slightly different from other sub-sections of this chapter, in which all GoF approaches and all alternative approaches in turn were discussed.) The sub-section concludes with an assessment of the contribution of GoF approaches to the development of broad-spectrum vaccines and therapeutics.

15.1.5.1 Development of New Coronavirus Vaccines

Currently, there are no FDA-approved vaccines for CoVs, which represents a critical gap in our public health preparedness for CoV outbreaks.

15.1.5.1.1 Developing New Vaccine Platforms

Live Attenuated Vaccines Developed Using GoF Approaches

GoF approaches have the potential to benefit two aspects of the development of live attenuated vaccine (LAV) platforms, which is a type of vaccine that is being actively researched for its potential as a CoV vaccine platform. First, GoF approaches can inform the development of candidate LAV strains, which exhibit attenuated virulence relative to parental strains. Specifically, one strategy for generating LAV strains is through serial passaging in a non-human host (either an animal or cells derived from an animal), as adapting a virus to a new host typically attenuates the virus in humans (i.e., alters rather than enhances host tropism). Because this approach **alters host tropism**, it is considered to be a GoF approach under the NSABB Framework. Although serial passaging has been used historically for developing polio, smallpox and other viral vaccines, the approach has not been utilized for the purpose of developing CoV vaccine strains.[1278]

Another strategy for developing attenuated vaccine strains is through targeted mutagenesis to attenuate or knock out the function of known virulence factors. As discussed above, GoF studies seeking to develop strains with **enhanced virulence** represent the most efficient and effective strategy for identifying CoV virulence factors, though LoF approaches may also be used. For the purpose of developing LAV strains, one benefit of LoF approaches is that the experiment may directly generate an attenuated strain. In

[1278] Ulmer JB *et al* (2006) Vaccine manufacturing: challenges and solutions. *Nature biotechnology* 24: 1377-1383

contrast, GoF approaches that lead to the identification of virulence factors require follow-up studies to determine how to attenuate that factor or to render it non-functional. Nonetheless, given that few virulence factors have been identified in SARS/MERS, GoF methods currently represent the most efficient and viable approaches to inform the development of LAV strain candidates through targeted genetic modification.

Once a candidate LAV strain has been generated, the strain is typically serially passaged *in vitro* or *in vivo* to determine whether the virus recovers fitness/virulence, which represents a GoF approach by **enhancing its fitness in culture or virulence *in vivo***. Because a tendency to revert or acquire compensatory mutations that enhance fitness/virulence could seriously compromise the safety of a live attenuated vaccine, demonstrating the genetic stability of a candidate LAV is a critical aspect of its development. The rationale behind this concern is evidenced by the example of a candidate LAV strain for SARS, which was attenuated through targeted mutagenesis to disrupt the ion channel activity of the SARS E protein. Upon passaging in cell culture and in mice, the mutant virus acquired compensatory mutations that restored both ion channel activity and virulence, highlighting the risks associated with live attenuated vaccines.[1279] There are no alternative approaches that can provide this information.

Live attenuated vaccines are an appealing type of vaccine for CoVs for several reasons, including the fact that they mimic the natural infection cycle better than other types of vaccines, which may induce stronger and more protective immune responses, and that they can be administered in the same way that natural infections are acquired to trigger mucosal immunity, which is difficult to generate but is an important objective for achieving long-term protection against mucosal pathogens such as CoVs.[1280, 1281] Two different LAV candidates for SARS have been shown to completely protect against lethal virus challenge in mice, demonstrating the promise of this type of vaccine for CoVs.[1282,1283] The main concern associated with LAVs is their potential to regain virulence in people, especially elderly and immunocompromised people, who are important target groups for CoV vaccines due to their increased susceptibility to severe infection.[1284]

Alternative Types of Vaccines That Do Involve GoF for Their Initial Development

Several other types of CoV vaccines are in development, which do not rely on GoF approaches for their initial development, including inactivated whole virus vaccines, recombinant vaccines, DNA vaccines, viral vector-based vaccines, and virus-like particles (VLPs).[1285] Many of these vaccine types have shown promise, and each has strengths and limitations relative to the use of live attenuated vaccines. For example, DNA vaccines, which consist of plasmid DNA that encodes CoV proteins, are safe (because they do not contain infectious material) and are easy to design, stable, and inexpensive. However, DNA vaccines generally induce less protective immune responses than inactivated or live attenuated vaccines. Viral vector-based vaccines, which consist of a different virus (such as adenovirus) expressing a CoV

1279 Nieto-Torres JL *et al* (2014) Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS pathogens* 10: e1004077

1280 Zhang N *et al* (2014) Current advancements and potential strategies in the development of MERS-CoV vaccines. *Expert Rev Vaccines* 13: 761-774

1281 (2015b) Interviews with coronavirus researchers.

1282 Graham RL *et al* (2012) A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. *Nature medicine* 18: 1820-1826

1283 Fett C *et al* (2013) Complete protection against severe acute respiratory syndrome coronavirus-mediated lethal respiratory disease in aged mice by immunization with a mouse-adapted virus lacking E protein. *Journal of virology* 87: 6551-6559

1284 Zhang N *et al* (2014) Current advancements and potential strategies in the development of MERS-CoV vaccines. *Expert Rev Vaccines* 13: 761-774

1285 Ibid.

protein(s), elicit stronger immune responses than DNA vaccines but may cause harmful immune responses and inflammation.[1286,1287,1288]

The abilities and limitations of GoF and alt-GoF approaches to support the development of new CoV vaccines are summarized in Table 15.5. Taken together, both GoF and alt-GoF approaches contribute to the development of LAVs, and both LAVs and alternative vaccine platforms have shown promise. The type or types of vaccines that will ultimately prove to be most effective for SARS, MERS, and SARS/MERS-like coronaviruses is not yet clear based on vaccinology research conducted to date.[1289] Given the need for CoV vaccines, pursuing all promising strategies for vaccine development in tandem, including LAVs, will ensure that an effective vaccine is achieved in the shortest possible period of time.

[1286] Weingartl H *et al* (2004) Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. *Journal of virology* 78: 12672-12676

[1287] Deming D *et al* (2006) Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS medicine* 3: e525

[1288] Enjuanes L *et al* (2008) Vaccines to prevent severe acute respiratory syndrome coronavirus-induced disease. *Virus research* 133: 45-62

[1289] Zhang N *et al* (2014) Current advancements and potential strategies in the development of MERS-CoV vaccines. *Expert Rev Vaccines* 13: 761-774

Table 15.5. CoVs: Summary of the Benefits of GoF Approaches That Enhance Virulence in Mammals		
Benefits to Vaccine Development: Develop New Candidate Vaccines		
Approach	**Benefits**	**Limitations**
GoF Experimental Approaches: GoF Experimental Approaches: • Serial passaging of viruses in cells or animals [2] • Genetic modification to introduce genetic traits expected to enhance virulence [3]	Support development of LAVs, which have several advantages as a CoV vaccine platform • Most efficient and effective strategies for discovering novel viral virulence traits that are conserved across multiple virus strains, which may be good targets for attenuation for the development of candidate LAVs • Determine whether LAV candidates recover virulence upon passage in cells/animals, an important aspect of safety testing	• Cannot demonstrate that mutation or deletion of a given virulence factor is sufficient to attenuate viral replication and/or virulence • Concern that LAVs could recover virulence in people necessitates stringent safety testing
Alt-GoF approach #1: Alternative Experimental Approaches: • Genetic modification to introduce traits expected to attenuate virulence (Loss of Function) • Comparative sequence analysis of wild type strains with varied levels of virulence	• Can be used to demonstrate that mutating or deleting a viral virulence factor is sufficient to attenuate virus replication and/or virulence	• Limited utility for the discovery of novel viral factors that contribute to virulence, relative to GoF approaches
Alt-GoF approach #2: Alternative vaccine platforms that do not rely on GoF • Recombinant vaccines, DNA vaccines , and several others	• Many alternative vaccine platforms have shown promise for CoV vaccines	• Each alternative vaccine platform has a unique set of weaknesses relative to LAVs

15.1.5.1.2 Evaluating the Safety and Efficacy of New Vaccine Candidates

Ultimately, safety and efficacy testing of any vaccine must be conducted in an animal model that replicates human disease pathogenesis. As discussed above, the use of a pathogenesis model is critical for safety testing because pathogenesis models can reveal adverse side effects that replication models do not. Currently, the mouse-adapted SARS virus represents the best pathogenesis model for SARS-CoV infection. None of the current animal models for MERS replicate human disease pathology, and CoV researchers believe that adapting the virus for growth in mice, a GoF approach, is the most promising strategy for developing a pathogenesis model for MERS-CoV infection. Therefore, the development of animal-adapted viruses using GoF approaches is critical for the development of new CoV vaccines.

15.1.5.1.3 Summary – Benefits of GoF to CoV Vaccine Development, Relative to Alternative Approaches

Taken together, GoF approaches uniquely benefit several aspects of CoV vaccine development. First, GoF approaches involving the creation of strains with enhanced virulence represent the most efficient and effective strategy for identifying novel virulence factors to inform the development of candidate live attenuated vaccine strains, although LoF approaches can also be used and are critical for confirming that blocking the function of a virulence factor is sufficient to attenuate virulence. Second, GoF approaches (selecting for enhanced fitness/virulence) are uniquely capable of demonstrating whether LAV strains recover virulence upon growth *in vivo*, an important aspect of LAV safety. Finally, animal models developed using GoF approaches selecting for altered host range and enhanced virulence are critical for testing the safety and efficacy of any type of vaccine.

15.1.5.2 Development of New Coronavirus Therapeutics

Currently, there are no FDA-approved therapeutics for CoVs, which represents a critical gap in public health preparedness for CoV outbreaks.

The first step in the licensure process for new drugs involves submission of an Investigational New Drug (IND) application to the FDA's Center for Drug Evaluation and Research (CDER). CDER recommends that several types of nonclinical studies are conducted before starting Phase I clinical studies, including determination of the drug's mechanism of action, *in vitro* selection of resistant viruses to the investigational product, and the genotypic and phenotypic characterization of resistant viruses.[1290] Mechanism of action studies should demonstrate the investigational product's ability to specifically inhibit viral replication or virus-specific function and should establish the site of the product's action.

GoF approaches have the potential to directly benefit several aspects of therapeutic development: (1) the identification of new therapeutic targets, (2) the determination of a drug's mechanism of action and the *in vitro* selection of resistant viruses, to support an IND application, and (3) the determination of dosing and/or combination therapies that are least likely to lead to emergence of resistance.

15.1.5.2.1 Identifying New Therapeutic Targets

CoV researchers cited the lack of knowledge of good viral targets for therapeutics as a critical limitation for the development of CoV therapeutics.[1291] As viral virulence factors are potentially good therapeutic

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[1290] Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

[1291] (2015b) Interviews with coronavirus researchers.

targets, GoF approaches that **enhance virulence** in cell culture or animal models have the potential to benefit the development of therapeutics by enabling the identification of new virulence factors. As discussed above, although alt-GoF approaches such as comparative analysis of the sequences of SARS epidemic strains or LoF approaches may also lead to the identification of viral proteins that contribute to virulence, GoF approaches currently represent the most efficient and effective way to identify novel virulence factors and gain insight into their mechanism of activity, a foundation for the development of antivirals. Ideally, researchers will identify conserved virulence factors that can be targeted by broad-spectrum therapeutics or using therapeutic platforms that can be readily adapted for emerging CoVs. Whether such virulence factors exist is not yet known, and additional research to identify and characterize the virulence factors of SARS, MERS, and SARS/MERS-like progenitor CoVs is needed to determine the feasibility of this approach. Notably, LoF approaches are needed to determine whether inhibiting or attenuating the function of a virulence factor is sufficient to reduce viral replication and/or infection-associated pathology during infection.

An alternative approach to the targeted development of therapeutics involves high-throughput screening of compounds for their ability to reduce viral replication *in vitro*.[1292,1293,1294,1295,1296] This is also an active area of therapeutic research in the CoV field and has generated several promising candidates. One drawback of this approach is that it is limited to the identification of compounds that reduce viral replication, which is only one aspect of virulence. Targeting other aspects of virulence, such as viral interactions with the host immune system, may prove to be a more effective therapeutic strategy. A related alternative approach involves high-throughput screening of panels of monoclonal antibodies (mAbs) to identify mAbs that bind to CoV Spike proteins, as mAbs targeting the Spike protein have been shown to effectively prevent viruses from infecting cells and could prime the immune system to clear the infection.[1297] One potential drawback of this therapeutic strategy is that CoVs can readily acquire mutations in their Spike protein that enable escape from mAb neutralization; however, researchers are actively pursuing the development of "cocktails" of mAbs that are more robust to the generation of escape mutants.[1208,1299] Additional drawbacks are that antibody-based therapeutics, which are uncommon for infectious diseases, may only slow infections and must be injected because antibodies are not small molecules.

The strengths and weaknesses of GoF and alt-GoF approaches for informing the development of new CoV therapeutics are summarized in Table 15.6. Taken together, both GoF and alt-GoF approaches represent promising strategies for the development of candidate therapeutics. The types of therapeutics that will ultimately prove to be most effective for SARS, MERS, and SARS/MERS-like coronaviruses is not yet clear based on therapeutic research conducted to date.[1300] Given the need for CoV therapeutics,

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1292 de Wilde AH *et al* (2014) Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrobial agents and chemotherapy* 58: 4875-4884

1293 Dyall J *et al* ibid Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. 4885-4893

1294 Ratia K *et al* (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences of the United States of America* 105: 16119-16124

1295 Wu CY *et al* (2004) Small molecules targeting severe acute respiratory syndrome human coronavirus. Ibid. 101: 10012-10017

1296 Severson WE *et al* (2007) Development and validation of a high-throughput screen for inhibitors of SARS CoV and its application in screening of a 100,000-compound library. *Journal of biomolecular screening* 12: 33-40

1297 Sui J *et al* (2008) Broadening of neutralization activity to directly block a dominant antibody-driven SARS-coronavirus evolution pathway. *PLoS pathogens* 4: e1000197

1298 Rockx B *et al* (2010) Escape from human monoclonal antibody neutralization affects in vitro and in vivo fitness of severe acute respiratory syndrome coronavirus. *The Journal of infectious diseases* 201: 946-955

1299 Sui J *et al* (2014) Effects of human anti-spike protein receptor binding domain antibodies on severe acute respiratory syndrome coronavirus neutralization escape and fitness. *Journal of virology* 88: 13769-13780

1300 (2015b) Interviews with coronavirus researchers.

pursuing all promising strategies for therapeutic development in tandem will ensure that an effective vaccine is achieved in the shortest possible period of time.

Table 15.6. CoVs: Summary of the Benefits of GoF Approaches That Enhance Virulence in Mammals		
Benefits to Therapeutic Development: Develop New Candidate Therapeutics		
Approach	**Benefits**	**Limitations**
GoF Approach #1: GoF Experimental Approaches: • Serial passaging of viruses in cells or animals [2] • Genetic modification to introduce genetic traits expected to enhance virulence [3]	• Most efficient and effective strategies for discovering novel viral virulence traits that are conserved across multiple virus strains, which may be good targets for new therapeutics	• Cannot demonstrate that inhibition of a given virulence factor is sufficient to attenuate disease pathogenesis
Alt-GoF approach #1: Alternative Experimental Approaches: • Genetic modification to introduce traits expected to attenuate virulence (Loss of Function) • Comparative sequence analysis of wild type strains with varied levels of virulence	• Can be used to demonstrate that blocking or attenuating the function of a viral virulence trait is sufficient to attenuate disease pathogenesis	• Limited utility for the discovery of novel viral factors that contribute to virulence, relative to GoF approaches
Alt-GoF approach #2: High-throughput screening of small molecule compounds to identify those that inhibit viral replication *in vitro*	• Approach has generated several promising therapeutic candidates	• Limited to the discovery of compounds that inhibit viral replication, which is only one aspect of pathogenesis
Alt-GoF approach #3: Identify neutralizing monoclonal antibodies (mAbs) targeting the CoV Spike protein	• Approach has generated several promising therapeutic candidates	• CoVs can readily acquire mutations that confer resistance to neutralization by a given mAb • mAb-based therapeutics have several drawbacks, including high production costs and the need for injection-based delivery

15.1.5.2.2 Determining the Mechanism of Antiviral Activity of a Therapeutic

The FDA recommends that a drug's mechanism of action be "well-characterized" prior to the start of Phase I clinical trials and requests this information as a component of an IND application, the first step of the licensing process.[1301] As discussed above, the CoV field is currently pursuing three strategies for drug development: (1) the deliberate targeting of known virulence factors or virulence pathways, (2) high-throughput screening of panels of mAbs (either derived from convalescent patient sera or from libraries of *de novo* generated mAbs) to identify mAbs that bind to CoV Spike proteins, and (3) high-throughput screening of FDA-approved drugs to identify therapeutics that inhibit viral replication *in vitro*. In the first two cases, the viral target of the therapeutic may be known, whereas in the last case, the target of the therapeutic is unknown, including whether the therapeutic targets the virus or the host. GoF approaches can be used to gain insight in the mechanism of activity of a therapeutic, thus benefitting the development of new drugs. Here the benefit of GoF approaches, relative to alternative experimental approaches, for the determination of antiviral mechanisms in both of these scenarios is evaluated.

GoF Approaches – Benefits and Limitations

Passaging viruses in cells in the presence of a therapeutic is a classic method for generating viruses that can **evade the inhibitory action of the therapeutic**, thus constituting a GoF approach. Viruses are then sequenced to identify mutations that arose, and if multiple mutations are present, mutations are re-introduced into the parental strain individually and in combination to identify the minimal set of mutation(s) that are necessary and sufficient to confer antiviral resistance. Understanding which viral protein or proteins mutate in order for the virus to escape inhibition suggests those proteins are targeted by the therapeutic, and the site and phenotypic consequences of the mutations may provide insight into the mechanism of antiviral activity. Together, this information provides a foundation for follow-up structural, biochemical, and cell biological assays investigating the mechanism of antiviral activity. A major strength of this approach is that it can be applied to any type of therapeutic, including therapeutics with known targets (but unknown mechanisms of action) and therapeutics with unknown targets. However, elucidating the mechanisms of antiviral activity based on indirect observations about antiviral resistance can be challenging. For example, mutations may arise in proteins that are not directly targeted by the therapeutic, or the phenotypic consequences of mutations may be unclear.[1302,1303,1304] Additionally, if the drug targets a host protein, this approach provides indirect information about its mechanism of activity, which must be inferred based on prior knowledge of virus-host interactions.

Alternative Approaches – Benefits and Limitations

Therapeutic candidates that are identified through high-throughput screens may attenuate viral replication by directly targeting viral proteins or by indirectly targeting host proteins. For that reason, emergence of resistance studies, which investigate potential viral targets, are usually complemented by high-throughput RNAi screens targeting host proteins, to investigate potential host targets. Specifically, the fact that knockdown of a particular host protein impedes the drug's ability to inhibit viral replication suggests that that protein or that signaling pathway may be targeted by the therapeutic. Though an informative strategy

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[1301] Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

[1302] Wensing AM *et al* (2014) 2014 Update of the drug resistance mutations in HIV-1. *Topics in antiviral medicine* 22: 642-650

[1303] Staschke KA *et al* (1995) Molecular basis for the resistance of influenza viruses to 4-guanidino-Neu5Ac2en. *Virology* 214: 642-646

[1304] Blick TJ *et al* (1998) The interaction of neuraminidase and hemagglutinin mutations in influenza virus in resistance to 4-guanidino-Neu5Ac2en. Ibid. 246: 95-103

for the study of therapeutics targeting host proteins, high-throughput RNAi screens provide minimal information about potential viral targets of therapeutics. Viral targets must be inferred based on prior knowledge of virus-host interactions, which is likely to be challenging given that current knowledge about CoV-host interactions is limited. Furthermore, because this kind of indirect information does not provide insight into antiviral mechanisms, this host-focused approach is of limited value for the study of therapeutics with known viral targets.

If the therapeutic target of a drug is known, analyzing the crystal structure of the viral target in complex with the antiviral compound (or mAb) can provide insight into the compound's mechanism of activity.[1305,1306] This approach is particularly useful for therapeutics that directly bind to and inhibit the activity of a viral protein. Though X-ray crystallography is appealing for its potential to provide direct information about the interaction between an antiviral and its target, inferring how that interaction affects a process in the viral life cycle may be difficult from such a static snapshot. In addition, this approach is less suitable for investigating therapeutics that target a protein-protein or protein-nucleic acid complex (either a virus-host complex or a virus-virus complex), either to inhibit the function or block the formation of the complex. The relevant interaction partner may be unknown, or recombinantly producing and crystallizing the protein complex may be difficult. Critically, because of the high level of effort required for X-ray crystallography, it is not a feasible approach for simply screening the potential viral targets of an unknown antiviral.

Photoaffinity cross-linking represents an alternative approach for identifying the binding site of a drug with a known target. In brief, this approach relies on the use of a "photoaffinity analogue" of the candidate therapeutic, which is synthesized to contain a photosensitive group (e.g., an azide) and a radioactive isotope (e.g., tritium, ^{3}H).[1307] After treating the viral protein with the photoaffinity analog, the sample is irradiated with UV light, triggering the photosensitive group to form a covalent bond with the viral enzyme. Analytical techniques such as mass spectrometry can then be used to identify the labeled amino acid residues in order to determine the drug's binding site. This technique shares strengths and weaknesses with X-ray crystallography. Namely, photoaffinity cross-linking is useful for small molecule drugs that directly bind to and inhibit the activity of a viral protein and does not require prior knowledge of the location of the drug binding site.[1308] However, inferring the mechanism of antiviral activity based on knowledge about the drug-virus protein interaction may be difficult, and the approach is less suitable for studying therapeutics that target a protein-protein or protein-nucleic acid complex (either a virus-host complex or a virus-virus complex).

Summary – Benefits of GoF Approaches Relative to Alternative Approaches

The strengths and limitations of GoF and alt-GoF approaches that can provide insight into the mechanism of action of a new therapeutic are summarized in table 15.7. Taken together, serial passaging of a virus in the presence of therapeutic to discover mutations that confer resistance, a GoF approach, is uniquely capable of identifying the viral target of a novel therapeutic with an unknown mechanism of action. For therapeutics with known viral targets, this information about resistance mutations can provide foundational information to guide follow-up structural, cell biological, and biochemical studies investigating the mechanism of action of the therapeutic. Although crystallography and photoaffinity

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1305 Prabakaran P *et al* (2006) Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. *The Journal of biological chemistry* 281: 15829-15836

1306 Ratia K *et al* (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences of the United States of America* 105: 16119-16124

1307 Cohen KA *et al* (1991) Characterization of the binding site for nevirapine (BI-RG-587), a nonnucleoside inhibitor of human immunodeficiency virus type-1 reverse transcriptase. *The Journal of biological chemistry* 266: 14670-14674

1308 Hamouda AK *et al* (2014) Photoaffinity labeling of nicotinic receptors: diversity of drug binding sites! *Journal of molecular neuroscience : MN* 53: 480-486

cross-linking can also provide insight into the antiviral mechanisms of therapeutics that directly bind to and inhibit virus proteins, inferring mechanistic information based on static information about the virus-antiviral complex may be difficult. Finally, the identification of host factors that are required for antiviral activity is a critical aspect of examining therapeutics with unknown targets. Though solely using host-focused approaches to elucidate the antiviral mechanism of a therapeutic that targets the virus would be difficult, this information complements GoF approaches to strengthen the evidence base for the drug's mechanism of action.

Table 15.7. Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Benefits to Therapeutic Development: Identify the Mechanism of Action of a Candidate Therapeutic

Approach	Benefits	Limitations
GoF Approach #1: Serial passaging of viruses in the presence of therapeutic [8]	• Identify the *viral* protein target of a candidate therapeutic with an unknown target • Provide insight into the mechanism of action of the therapeutic through the identification of mutations that confer resistance	• Elucidating the mechanism of action of a therapeutic based on indirect information about resistance mutations may be difficult o Resistance mutations may arise in non-target proteins, confounding interpretation of results • Not suitable for identifying the targets of therapeutics that target host proteins
Alt-GoF Approach #1: RNAi screen targeting host proteins to identify host proteins that are critical for the antiviral activity of a therapeutic	• Identify the *host* protein target of a candidate therapeutic with an unknown target	• Provides indirect information about the viral protein targets of a therapeutic
Alt-GoF Approach #2: Analyze the crystal structure of a therapeutic in complex with its viral protein target	• Provides direct information about the interaction between a therapeutic and its viral protein target o May provide insight into the mechanism of antiviral activity	• Limited to the study of therapeutics with known targets • Inferring mechanism of activity based on static information about the therapeutic-viral protein interaction may be difficult • Approach may not be suitable for the study of therapeutics that target protein-protein protein-nucleic acid complexes
Alt-GoF Approach #3: Photo-affinity crosslinking	• Provides direct information about the binding site of a therapeutic on its viral protein target o May provide insight into the mechanism of antiviral activity	• Limited to the study of therapeutics with known targets • Inferring mechanism of activity based on static information about the therapeutic binding site may be difficult • Approach may not be suitable for the study of therapeutics that target protein-protein protein-nucleic acid complexes

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental Information).*

15.1.5.2.3 Determining the Genetic Threshold for Resistance Development

Prior to the conduct of clinical trials and to support an IND application, the FDA recommends conducting *in vitro* studies for **selection of resistance to a therapeutic** in order to determine the genetic threshold for resistance development (i.e., how many mutations are needed to acquire resistance). Specifically, the FDA recommends passaging the virus in the presence of therapeutic, followed by sequencing of emergent resistant viruses and phenotypic characterization of resistant viruses.[1309] Selection for resistance studies should be repeated multiple times to determine if the same or different patterns of resistance mutations develop, as well as to determine how the concentration of the therapeutic impacts how readily resistance develops. These studies constitute GoF approaches. The FDA guidance does not suggest any alternative approaches that could provide similar information. In fact, prior to deployment of the therapeutic and the emergence of resistant viruses in nature, no alternative approaches can provide this information. Thus, GoF approaches that lead to the generation of viruses that are resistant to therapeutics in development are **essential** for the licensing of new therapeutics.

15.1.5.2.4 Determining the Therapeutic Dosage and/or Combination Therapies That Are Least Likely to Lead to the Emergence of Resistance

The therapeutic regimen, including therapeutic dose and the use of combination therapies, may influence whether and how readily antiviral resistance arises. In the context of candidate CoV therapeutics, combination therapies are relevant for the development of mAb-based therapeutics. Although mutations that prevent mAb binding may readily arise in the presence of a single mAb, acquiring mutations that confer resistance to multiple mAbs that target different sites on a virus protein may be difficult without compromising viability.[1310]

GoF approaches that lead to the development of viruses with **resistance to therapeutics in development** can be used to evaluate the relationship between emergence of resistance and therapeutic dosage or the administration of multiple therapeutics in combination. First, serial passaging of virus in animals dosed with varying amounts of the therapeutic provides insight into the dose-dependence of the emergence of resistant viruses. Because host-dependent factors, such as the rate of metabolism or clearance of the therapeutic, influence the concentration of therapeutic the virus experiences, conducting passaging studies in animals provides more relevant information than *in vitro* passaging studies. Second, serial passaging of virus in cells or in animals in the presence of multiple mAbs (or other types of therapeutics) can be used to determine how readily resistance arises in response to combination versus single therapies. Although *in vitro* selection studies are useful for screening different combinations of therapeutics, because of the role of bioavailability and other host-dependent factors on antiviral efficacy, all promising combination therapies should be validated through *in vivo* passaging experiments. No alternative approaches are capable of providing similar information about the dose-dependence of resistance or whether combination therapies lead to resistance less readily than individual therapies.

Taken together, GoF approaches that lead to the generation of viruses that are resistant to therapeutics in development are uniquely capable of determining the therapeutic dose that is least likely to lead to the acquisition of antiviral resistance as well as determining whether combination therapies better prevent the

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[1309] Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

[1310] Rockx B *et al* (2010) Escape from human monoclonal antibody neutralization affects in vitro and in vivo fitness of severe acute respiratory syndrome coronavirus. *The Journal of infectious diseases* 201: 946-955

emergence of resistant viruses than individual therapies. Both types of information benefit the development of therapeutic strategies that will be effective for a longer period of time in the field.

15.1.5.2.5 Efficacy Testing for Regulatory Approval

Currently, several animal models are available for the testing of SARS-CoV therapeutics: mouse-adapted strains (GoF), transgenic mice that have been sensitized to SARS infection through expression of the human ACE2 receptor (alt-GoF), and naturally susceptible species such as mice and ferrets. The mouse-adapted strains represent the only animal model system for SARS that replicates human disease pathology and thus provides a much more robust system for demonstrating the safety and efficacy of therapeutic candidates than other model systems. Additionally, mouse-adapted SARS strain may facilitate the licensing of therapeutics under the FDA's Animal Efficacy rule, which states that therapeutics against rare, emerging, or virulent agents such as SARS-CoV can achieve regulatory approval provided efficacy is demonstrated in multiple animal models that display clinical illness representative of human disease.[1311]

Two types of animal models are available for MERS: naturally susceptible hosts, such as rabbits, and transgenic animals that have been sensitized to MERS infection through expression of the human DPP4 receptor. None of the model systems that have been developed in either category replicate human disease pathology. Although these systems can be used to demonstrate that MCMs diminish viral replication, the relevance of results to human disease is uncertain, and these models cannot establish whether a therapeutic candidate is likely to reduce disease-associated pathology in humans. For that reason, researchers are actively pursuing the development of a mouse-adapted MERS strain through serial passaging approaches (GoF), which is thought to be the most promising strategy for developing a pathogenesis model for MERS-CoV infection that is suitable for advanced MCM testing.

Taken together, GoF approaches, namely serial passaging to develop animal-adapted strains that recapitulate human disease pathology during infection, are critical for testing the safety and efficacy of therapeutic candidates, thereby advancing therapeutic development.

15.1.5.3 Development of Broad-Spectrum Vaccines and Therapeutics

Although SARS-CoV is no longer circulating in nature, surveillance efforts over the past decade have revealed that SARS-CoV and MERS-CoV emerged from a reservoir of thousands of bat CoVs, many of which are genetically similar to SARS-CoV and MERS-CoV.[1312,1313] One SARS-like bat CoV was recently shown to be naturally capable of infecting human cells, suggesting that SARS/MERS-like bat CoVs have the potential to spill over into human populations.[1314] CoV researchers hypothesize that additional animal CoVs will emerge to cause epidemics because changing population patterns increasingly support the ability of CoVs to cause disease and spread in human populations. Namely, both crowding, which facilitates large respiratory droplet transmission of CoVs, and elderly populations, who are more susceptible to severe infection and death than younger age groups, are increasing worldwide.[1315] For that reason, CoV researchers are strongly interested in developing broad-spectrum vaccines and therapeutics that will be capable of targeting the next emerging CoV.

[1311] Food and Drug Administration. Guidance for Industry: Product Development Under the Animal Rule. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm399217.pdf. Last Update May 2014. Accessed 14 October 2015.

[1312] Vijaykrishna D *et al* (2007) Evolutionary insights into the ecology of coronaviruses. *Journal of virology* 81: 4012-4020

[1313] Graham RL *et al* (2013) A decade after SARS: strategies for controlling emerging coronaviruses. *Nature reviews Microbiology* 11: 836-848

[1314] Ge XY *et al* (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538

[1315] (2015b) Interviews with coronavirus researchers.

15.1.5.3.1 GoF Approaches – Benefits and Limitations

The generation of chimeric bat-SARS viruses through recombinant methods ("Spike swapping"), considered a GoF approach because the **host tropism of the chimeric virus may be altered** relative to that of the parental viruses, has the potential to benefit the development of broad-spectrum MCMs. Specifically, chimeric viruses are used as challenge viruses to explore the broad-spectrum potential of candidate vaccines and therapeutics, in order to test whether MCMs designed to target SARS/MERS proteins are also capable of targeting cognate proteins in bat CoVs as well as whether MCMs can target SARS/MERS proteins in a different virus context (representative of the next emerging CoV capable of infecting humans). These experiments can provide insight into whether MCMs targeting any CoV protein or process are capable of conferring broad-spectrum protection against bat CoVs with zoonotic potential, in addition to SARS and MERS. The major drawback of this approach is that results using artificial chimeric viruses may not reflect the capacity of MCMs to target the wild type viruses.

15.1.5.3.2 Alt-GoF Approaches – Benefits and Limitations

Several alternative approaches can be used to evaluate the broad-spectrum potential of candidate vaccines and therapeutics. One approach involves the use of wild type bat CoVs as challenge viruses, in lieu of chimeric bat-SARS viruses. However, the fact that few bat CoVs can be grown in culture or in animals without the use of GoF approaches (serial passaging or the generation of chimeric viruses) diminishes the utility of this approach.

For evaluating vaccines or monoclonal antibody therapies that target the Spike protein, the use of pseudotyped viruses represents another alternative approach. Because Spike proteins are presented differently in the context of pseudotyped viruses versus CoVs, especially quaternary epitopes that are critical for the specificity of Spike-antibody interactions, results using pseudotyped viruses may not be recapitulated in the context of the wild type virus.[1316] For example, researchers reported that certain mAbs that do not neutralize the wild type SARS virus are capable of neutralizing viruses that are pseudotyped with SARS Spike proteins.[1317] Thus, all results using pseudotyping systems must be confirmed using wild type viruses (or chimeric CoVs, which better mimic wild type bat CoVs than pseudotyped viruses).

Finally, chimeric viruses that have been engineered to express "internal" (i.e., non-Spike) CoV proteins have been used for testing the efficacy of MCMs targeting non-Spike proteins.[1318] As with pseudotyped viruses, due to significant differences in the course of infection between chimeric virus systems and wild type viruses, such chimeric virus systems can be used to screen therapeutic candidates but do not replace the need to test MCMs against the wild type virus.

15.1.5.3.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The strengths and limitations of model systems that can be used for the development of broad-spectrum CoV MCMs are summarized in Table 15.8. Taken together, chimeric bat-SARS CoV strains created using GoF approaches that **adapt a virus to a new host** are **uniquely capable** of providing reliable information about the broad-spectrum potential of CoV vaccines and therapeutics. Because most bat CoV strains cannot be cultured, the use of wild type viruses cannot provide information about whether CoV MCMs are capable of targeting a variety of SARS/MERS-like CoVs in addition to SARS and MERS. While expressing CoV proteins in the context of other viruses (i.e., pseudotyped viruses and other chimeric virus systems) may be useful for screening MCM candidates, all results must be confirmed using wild type

[1316] Ibid.
[1317] Ibid.
[1318] Deng X *et al* (2014) A chimeric virus-mouse model system for evaluating the function and inhibition of papain-like proteases of emerging coronaviruses. *Journal of virology* 88: 11825-11833

strains (or CoV chimeric strains) due to significant differences in the behavior of chimeric viruses versus CoVs.

Table 15.8. Comparison of Model Systems for the Development of Broad-Spectrum Vaccines and Therapeutics

Model system	Benefits	Limitations
GoF: "Spike swapping" - chimeric CoVs • Animal CoV plus SARS Spike [5] • SARS plus animal CoV Spike [6]	• Enables testing of whether MCMs targeting any CoV protein or process confer broad-spectrum protection against multiple animal CoVs • Use of chimeric CoVs is more relevant to nature than using mixed virus chimeras	• Results using chimeric viruses may not reflect the capacity of MCMs to target wild type viruses
Alt-GoF #1: Wild type animal CoVs	• Use of wild type viruses is most relevant to nature	• Most wild type animal CoVs cannot be grown in culture
Alt-GoF #2: Pseudotyped viruses – express CoV Spike proteins in the context of a different virus	• Enables testing of whether MCMs targeting the Spike protein confer broad-spectrum protection against multiple animal CoVs	• Results may not be recapitulated in the context of the wild type virus o Differential presentation of the Spike protein on the virus surface influences antibody binding
Alt-GoF #3: Other mixed virus chimeras • Express "internal" (non-Spike) CoV proteins in other viruses	• Enables testing of whether MCMs targeting non-Spike proteins confer broad-spectrum protection against multiple animal CoVs	• Results may not be recapitulated in the context of the wild type virus o Different course of infection and expression levels of CoV proteins affect therapeutic efficacy

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental Information).*

15.2 Influenza Viruses: Detailed Analysis of the Benefits of GoF Research that Enhances Virus Production

15.2.1 Overview of the GoF Landscape: Approaches that Enhance the Production of Influenza Viruses

This assessment describes the benefits of GoF experimental approaches that are reasonably anticipated to enhance the production of influenza viruses. In this section, an overview of GoF approaches in this phenotypic category is provided and the scientific outcomes and/or products of each approach are described.

15.2.1.1 Generation of Attenuated, High-Yield Candidate Vaccine Viruses Through Reassortment

Reassortment between a wild type strain and an attenuated, high-yield vaccine backbone strain generates a "Candidate Vaccine Virus" (CVV), which comprises the HA and NA genes from the wild type strain and the remaining six "internal genes" from the vaccine backbone strain. CVVs are attenuated and exhibit higher levels of growth relative to the parental, wild type virus. CVVs may be generated through classical reassortment methods, which involve co-infection of eggs or cells with the wild type strain and the vaccine backbone strain followed by antibody-based selection for viruses with the correct surface antigens, or through reverse genetics.[1319] CVVs serve as the basis of vaccine strains that are used for the production of influenza vaccines in eggs or cells. Additionally, in the context of academic research, comparing the sequences of CVVs with varied growth properties enables the identification of mutations that are associated with high yield.

15.2.1.2 Serial Passaging of Viruses in Eggs or Cells

Serial passaging of viruses in eggs or cells selects for higher-yield viruses. This approach is currently used for the production of influenza vaccines in eggs or cells as well as for basic science research on the mechanisms underlying high growth of influenza vaccine viruses. For vaccine production, manufacturers serially passage CVVs in eggs or cells to generate high-yield vaccine seed strains that can be used for large-scale production of vaccines. In the context of academic research, serial passaging of viruses in eggs or cells followed by sequencing of the emergent higher-yield viruses enables the identification of mutations that are sufficient to enhance the growth of the viruses. Subsequently, mutant viruses are subjected to antigenic characterization using the hemagglutinin inhibition (HAI) assay or other assays to identify which mutations confer high growth without changing the antigenicity of the strain. For research purposes, this approach is most commonly carried out using vaccine backbone strains and CVVs but may also be carried out using wild type strains.

15.2.1.3 Forward Genetic Screen to Identify Mutations That Confer High Growth to Viruses

Forward genetic screens, which involve random mutagenesis of viruses followed by limited passaging to select for mutants with high growth properties, enable the identification of mutations that confer high growth to viruses. Forward genetic screens involving vaccine backbone strains and CVVs lead to the identification of mutations that are sufficient to enhance the yields of vaccine viruses. Subsequently, mutant viruses are subjected to antigenic characterization using the hemagglutinin inhibition (HAI) assay

[1319] Use of classical reassortment methods to generate CVVs may lead to the generation of a 5:3 reassortment strain which includes the HA, NA, and one additional gene from the wild type strain and the remaining five genes from the vaccine backbone strain.

or other assays to determine which mutations confer high growth without altering the antigenicity of the strain.

15.2.1.4 Targeted Mutagenesis of Viruses to Introduce Mutations That Are Associated with High Growth

Targeted mutagenesis of viruses to introduce mutations that are associated with high growth, followed by characterization of virus yields relative to the parental virus, demonstrates that a mutation or set of mutations is necessary and sufficient to confer high growth. Subsequently, antigenic characterization assays are performed to confirm that the mutations have not altered the antigenicity of the virus, and the mutant strain is subjected to several rounds of passaging in eggs or cells to ensure that it is genetically stable – that is, that it does not acquire additional mutations that alter its antigenicity upon further growth. This knowledge provides a foundation for follow-up studies investigating the mechanistic basis of the high-growth phenotype (e.g., the use of cell biological assays, biochemical assays, and other assays to explore how the mutation enhances growth). Notably, these mutations may have been discovered through a GoF approach, such as serial passaging or a forward genetic screen, or through an alt-GoF approach, such as comparative analysis of wild type sequences.

Finally, it should be noted that experimental approaches involving targeted genetic modification of the viral polymerase complex of avian viruses to render it more "human-like" (through site-directed mutagenesis or reassortment between human and avian viruses) is also likely to enhance virus replication. However, as the primary goal of those studies is to gain insight into the mechanisms underlying adaptation of avian viruses to mammals, those studies are discussed in Section 16.3 ("detailed analysis of the benefits of GoF research that enhances mammalian adaptation and transmissibility").

15.2.2 Overview of the Potential Benefits of GoF Approaches That Enhance the Production of Influenza Viruses

This section includes evaluation of whether GoF approaches that enhance virus production, described above, have the potential to benefit each of the general benefit areas described in the NSABB's "Framework for Conducting Risk and Benefit Assessments of Gain of Function Research." Each potential benefit will be evaluated in detail below.

15.2.2.1 Scientific Knowledge Benefits

Information about genetic traits that confer high growth to vaccine viruses provides a foundation for follow-up studies investigating the mechanistic basis of the enhanced growth phenotype, thereby benefiting scientific knowledge about mechanisms underlying the high growth of vaccine viruses.
It should be noted that this type of GoF research has a clear translational focus, in that these studies aim to learn how to modulate the phenotypic properties of attenuated, high-yield vaccine viruses rather than to gain insight into the natural behavior of wildtype viruses.

15.2.2.2 Surveillance

All other GoF approaches are focused on identifying mutations that confer high growth to vaccine viruses (either candidate vaccine viruses or vaccine backbone strains). Because these viruses have no correlate in nature, this information does not inform the interpretation of genetic surveillance data from animals or humans.

15.2.2.3 Development and Production of Vaccines

GoF approaches, namely the generation of attenuated, high-yield CVVs and serial passaging, are core aspects of the existing processes for the production of influenza vaccines in eggs and cells, thus these approaches currently benefit the production of influenza vaccines. The insights gleaned from GoF approaches that enhance virus production also have the potential to improve vaccine production practices in the future through two distinct mechanisms: (1) shortening vaccine production timelines, and (2) improving the match between the virus strains used as the basis of vaccine strains and the strains that are circulating during flu season (referred to as "vaccine match," which is correlated with vaccine efficacy). In brief, the former benefit derives from the creation of higher-yield vaccine viruses and the identification of genetic traits that confer high growth to vaccine viruses, and the latter benefit derives from the creation of genetically stable vaccine viruses that do not acquire antigenicity-altering mutations upon growth in eggs or cells.

15.2.2.4 Therapeutics and Diagnostics

Information about mutations that confer high growth to vaccine viruses or about mutations that rescue the growth of antiviral resistant strains is not relevant to the development of therapeutics.

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[1320,1321]

15.2.2.5 Informing Policy Decisions

Since information about compensatory mutations that rescue the growth of antiviral resistant strains does not inform assessments of the risk posed by circulating influenza strains, this information does not benefit policy decisions about public health preparedness.

Similarly, information about mutations that confer high growth to vaccine viruses does not inform the analysis of genetic surveillance data, so this information does not benefit policy decisions about public health preparedness.

15.2.2.6 Economic Benefits

Increasing the yields of vaccine viruses, using information or products derived from GoF approaches that enhance virus production, is likely to lower the cost per vaccine dose by enabling the production of a greater number of vaccine doses using the same quantity of input materials. The economic benefits of enhancements to vaccine virus yields were not described in detail in this report.

Because academic research investigating the mechanisms underlying high growth of vaccine viruses aims to generate information or products that can be applied to vaccine production in order to address shortcomings in the current process, first, an overview of existing systems for the production of influenza vaccines is provided, including the role of GoF approaches. Then, the benefit of GoF approaches that are currently used in influenza vaccine production for the availability and efficacy of influenza vaccines are evaluated. In this sub-section, given the continued need for production of new seasonal influenza

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[1320] New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

[1321] (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

vaccines, the potential for alternative approaches to provide the same or similar benefits in the immediate future are also evaluated.

Next, shortcomings in the existing process for influenza vaccine production are reviewed; this motivates the body of academic research that aims to improve the yields of vaccine viruses and the application of that research to vaccine production. Then the potential for GoF approaches to identify genetic markers of high growth and to advance foundational knowledge about mechanisms underlying high growth *in ovo* and in cell culture, relative to alternative experimental approaches is evaluated. Finally, the potential for the information/products derived from GoF research to further improve vaccine production practices, relative to alternative experimental approaches and alternative scientific/technical innovations that can similarly benefit the availability and efficacy of vaccines in the future, is evaluated.

15.2.3 Benefits of GoF Research that Enhances Production of Influenza Viruses to Current Vaccine Production Practices

15.2.3.1 Current Processes for Production of Influenza Vaccines

To provide context for the evaluation of the benefits of GoF approaches to the current production of influenza vaccines, first, a brief overview of existing influenza vaccine production processes is provided. This review also provides important context for the subsequent discussion of the potential benefits of GoF research to *future* vaccine production processes.

Because existing influenza vaccines rely predominantly on the immune response to the influenza HA protein and are strain-specific, there is a continued need for production of new influenza vaccines to protect public health. Specifically, seasonal influenza vaccines must be updated annually to accommodate antigenic drift of circulating influenza viruses, and specific vaccines must be produced in response to the emergence of a novel pandemic strain. Three different influenza vaccine production technologies have been approved by the Food and Drug Administration (FDA): egg-based vaccines, cell-based vaccines, and recombinant vaccines.[1322] Egg- and cell-based vaccines are derived from whole viruses, whereas recombinant vaccines are virus-free. The majority of influenza vaccines produced in the US are derived from viruses grown in embryonated chicken eggs. Egg-grown viruses may be chemically inactivated and delivered as a "flu shot," a method of vaccine production that has been used for over 70 years, or delivered as live attenuated vaccines in the form of a nasal spray.[1323,1324,1325] Recently, a process using cultured mammalian cells has been developed for the production of inactivated influenza vaccines; one cell-based vaccine has been commercially available in the US since 2012.[1326] Recombinant vaccines, which are virus-free vaccines that are based on influenza proteins produced in insect cells or other protein expression system, represent the newest production technology. One recombinant vaccine was FDA-approved in 2013, and several others are in various stages of commercial development.[1327,1328,1329]

[1322] How Influenza (Flu) Vaccines are Made. CDC. http://www.cdc.gov/flu/protect/vaccine/how-fluvaccine-made.htm. Last Update Accessed September 14, 2015.
[1323] Ibid.
[1324] Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*. 2nd Edition edn, pp 352-370.
[1325] TABLE. Influenza vaccines — United States, 2015–16 influenza season. http://www.cdc.gov/flu/protect/vaccine/vaccines.htm. Last Update Accessed September 14, 2015.
[1326] How Influenza (Flu) Vaccines are Made. CDC. http://www.cdc.gov/flu/protect/vaccine/how-fluvaccine-made.htm. Last Update Accessed September 14, 2015.
[1327] Ibid.
[1328] Bright R. Review of New Vaccine Platforms and Influenza Vaccine Pipeline. http://www.who.int/influenza_vaccines_plan/resources/bright.pdf. Last Update Accessed September 15, 2015.
[1329] Shaw A (2012) New technologies for new influenza vaccines. *Vaccine* 30: 4927-4933.

The processes and timelines for production of egg- and cell-based vaccines, including inactivated and live attenuated vaccines, are similar (Figure 15.1).

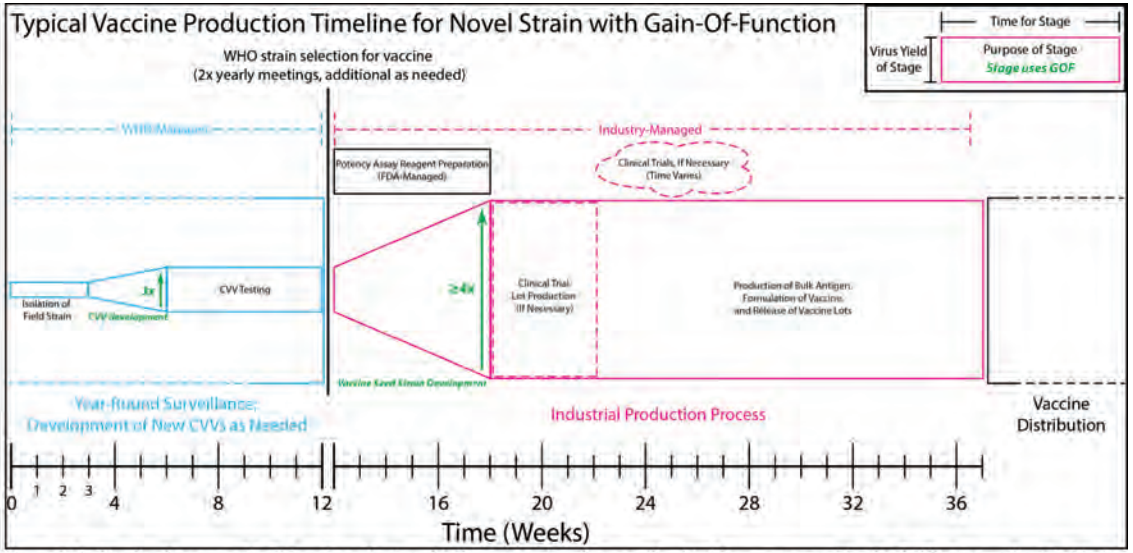

Figure 15.1. Timeline for egg- and cell-based production of influenza vaccines. Steps outlined in blue are managed by the WHO, steps outlined in black are managed by the FDA, and steps outlined in pink are managed by vaccine manufacturers. Two steps – candidate vaccine virus (CVV) development and vaccine seed strain development – involve GoF approaches and are highlighted in green text. The height of the bars reflects the yield of the virus at that stage; trapezoidal stages indicate that virus yields are enhanced over the course of that step. The length of the bars reflects the average time needed to complete that stage of the process. Clinical trials are not conducted for new seasonal influenza vaccines but are conducted for pandemic influenza vaccines; the scale of clinical trials and length of this stage will vary depending on the strain. Overall, production of multivalent seasonal or monovalent pandemic influenza vaccines spans six to eight months. For production of seasonal flu vaccines, this timeline begins with WHO strain selection (week 12 in the above timeline). For production of pandemic strains, this timeline begins with isolation of the field strain and CVV development (week 0 in the above timeline).

First, a selected field isolate that is representative of circulating strains must be attenuated and its growth in eggs/cells must be enhanced in order to be suitable for large-scale manufacturing of vaccine virus. This growth enhancement is achieved through the use of two different GoF approaches. The first GoF approach involves reassortment between a field isolate and an attenuated, high-yield "vaccine backbone strain" to generate a CVV, as described above.[1330] CVVs undergo a series of characterization assays before they are released to manufacturers, including pathogenicity testing in ferrets, antigenic characterization, and several rounds of passaging to ensure that mutations that lead to antigenic changes will not arise during growth in eggs/cells.[1331,1332,1333] Upon receipt of a CVV, vaccine manufacturers serially passage the CVV in eggs or cells to increase its yield, representing the second GoF approach used to enhance the yields of vaccine viruses during the vaccine production process.

Collectively, the result is a high-yield vaccine seed virus that can be used for large-scale production of vaccine virus. In parallel to vaccine seed strain development, the FDA prepares "potency reagents" for the single-radial immune-diffusion (SRID) assay used to standardize antigen quantities, namely HA antigen and HA-specific antiserum produced in sheep.[1334] Large-scale production of bulk antigen involves nested cycles of virus production in eggs or cells, purification and processing of virus (including chemical inactivation, if applicable), and quantification of HA antigen yields using the SRID assay. For production of seasonal, multivalent vaccines, vaccine doses are formulated following consecutive production of monovalent bulk antigen for each component of the vaccine (one A/H1N1 strain, one A/H3N2 strain, and one or two B strains).[1335,1336,1337] New seasonal vaccines are not clinically tested each year, but pandemic vaccines must undergo clinical trials to establish the safety of the vaccine and determine the dosing parameters needed to elicit a strong immune response (e.g., amount of antigen, number of doses, etc.). Manufacturers set aside an initial lot(s) of vaccine antigen for clinical trial use, and the trials are conducted in parallel with additional bulk antigen production.[1338,1339] Finally, all lots of seasonal and pandemic vaccines are safety-tested and FDA-approved prior to release.

Overall, the production of egg- and cell-based influenza vaccines requires six to eight months.[1340,1341] For production of pandemic vaccines, this timeline begins with the selection of a field isolate to be used as the

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1330 It should be noted that although CVVs are usually 6:2 reassortants (i.e.,comprising the HA and NA genes from the field isolate and all other genes from the vaccine backbone strain), CVVs may also be 5:3 reassortants (e.g. HA, NA, and other gene from the field isolate, and the remaining five genes from the vaccine backbone strain).

1331 Vaccine response to the avian influenza A(H7N9) outbreak- step 1: development and distribution of candidate vaccine viruses. http://www.who.int/influenza/vaccines/virus/CandidateVaccineVirusesH7N9_02May13.pdf. Last Update Accessed September 14, 2015.

1332 Update of WHO biosafety risk assessment and guidelines for the production and quality control of human influenza vaccines against avian influenza A(H7N9) virus. http://www.who.int/biologicals/areas/vaccines/influenza/biosafety_risk_assessment_10may2013.pdf. Last Update Accessed September 14, 2015.

1333 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1334 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

1335 Food and Drug Administration. Annex 5: Vaccination Development and Production - Draft. http://www.hsdl.org/?view&did=459937. Last Update Accessed September 15, 2015.

1336 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

1337 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1338 Ibid.

1339 Cho D. Regulatory Pathways for Registration of Seasonal and Pandemic Influenza Vaccines: FDA Approach. http://www.who.int/phi/Day2_2_Cho_FDA_approach_Flu_vax_PM_Dubai2013.pdf. Last Update 19 March 2013. Accessed 14 September 2015

1340 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1341 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

basis of the vaccine strain and includes the time needed for development and testing of the CVV. For seasonal influenza vaccines, production is initiated following strain selection by the WHO in February or September (for the Northern and Southern hemispheres, respectively). The WHO Global Influenza Surveillance and Response System (GISRS) oversees the development and testing of new CVVs throughout the year, when antigenically distinct strains emerge, and the strain selection committee recommends strains for which antigenically similar CVVs are available.[1342]

15.2.3.2 GoF Approaches Needed to Maintain Current Influenza Vaccine Production Systems

Because the strain composition of influenza vaccines must be updated annually, the CDC's Advisory Committee on Immunization Practices recommends annual influenza vaccination for all people ages six months and older.[1343] Currently, over 99% of influenza vaccines used in the US are produced in eggs or cells,[1344,1345] which relies on GoF approaches for two stages of the production process: CVV development and vaccine seed strain production (Figure 15.1). As described above, each of those GoF approaches enhances virus production, collectively increasing HA antigen yield at least 12-fold relative to the cognate wildtype strain.[1346] Altogether, these approaches, which are used throughout the egg- and cell-based vaccine manufacturing industry, result in the production of over 170 million doses of seasonal influenza vaccine annually.[1347] It should also be noted that attenuated, high-yield candidate vaccine viruses have been used for the production of influenza vaccines since 1971.[1348,1349,1350]

Because of the continued need for production of seasonal influenza vaccines, as well as the need to maintain robust capabilities for the production of pandemic vaccines for pandemic preparedness, alternative approaches must similarly benefit vaccine production in the immediate future. Eliminating GoF approaches from existing production processes would necessitate the use of vaccine viruses with wild type growth properties, which could be achieved through the direct use of field isolates or through the use of novel reassortants that are attenuated but do not exhibit enhanced yields (Table 15.9).

1342 (WHO) WHO. Recommended composition of influenza virus vaccines for use in the 2015- 2016 northern hemisphere influenza season. http://www.who.int/influenza/vaccines/virus/recommendations/201502_recommendation.pdf?ua=1. Last Update February 26, 2015. Accessed October 20, 2015.

1343 CDC's Advisory Committee on Immunization Practices (ACIP) Recommends Universal Annual Influenza Vaccination. http://www.cdc.gov/media/pressrel/2010/r100224.htm. Last Update Accessed September 15, 2015.

1344 Dowling B. Protein Sciences' N.Y. Factory Licensed For Flu Vaccine Production. http://www.courant.com/business/hc-protein-sciences-pearl-river-approval-20150513-story.html. Last Update 13 May 2015. Accessed 14 September 2015.

1345 CDC. What You Should Know for the 2015-2016 Influenza Season. http://www.cdc.gov/flu/about/season/flu-season-2015-2016.htm. Last Update Accessed September 15, 2015.

1346 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1347 CDC. What You Should Know for the 2015-2016 Influenza Season. http://www.cdc.gov/flu/about/season/flu-season-2015-2016.htm. Last Update Accessed September 15, 2015.

1348 Kilbourne ED (2006) Influenza pandemics of the 20th century. *Emerging infectious diseases* 12: 9-14.

1349 World Health Organization. Influenza vaccine viruses and reagents. http://www.who.int/influenza/vaccines/virus/en/. Last Update September 2015. Accessed 30 September 2015.

1350 Nesterova D. Influenza Vaccine History. http://www.vaccination.english.vt.edu/wp-content/uploads/2015/04/updated-influenza-media-kit-4.pdf. Last Update October 2012. Accessed 30 September 2015.

Table 15.9. Summary of the Benefits of GoF Approaches that Enhance Virus Production

Vaccine Development Benefits – Current Influenza Vaccine Production Practices			
Experimental Approach	**Benefits**	**Limitations**	**Barriers**
GoF [1 – 4]*: Use of high-growth reassortant strains for vaccine production (status quo), which exhibit: • Enhanced virus production • Attenuated virulence	• Annual production of > 170 million doses of seasonal influenza vaccine • Ability to release pandemic flu vaccine ~ 8 months after emergence of a novel pandemic strain	N/A (discussed elsewhere)	None (current system)
Alt-GoF #1: Use of wild type strains for vaccine production	• Avoid use of vaccine strains with enhanced yield relative to wild type viruses	• Adverse consequences for vaccine availability o Inability to produce vaccine that meets FDA purity standards o Significantly reduced rates of vaccine production • Adverse consequences for vaccine match o Prioritize growth properties over antigenic properties when choosing strains for vaccine production o Choose seasonal strains for vaccine at least one year in advance of the start of the target flu season	• Construction of new manufacturing facilities capable of large-scale production of wild type viruses that are pathogenic to humans
Alt-GoF #2: Use of novel reassortant strains that are: • Attenuated • Exhibit wild type levels of virus production	• Avoid use of vaccine strains with enhanced yield relative to wild type viruses		• Requires development of new vaccine backbone strains that are attenuated but do not confer high growth o Commercial use of new vaccine backbone strains may require FDA approval

Table 15.9. Summary of the Benefits of GoF Approaches that Enhance Virus Production			
Vaccine Development Benefits – Current Influenza Vaccine Production Practices			
Experimental Approach	**Benefits**	**Limitations**	**Barriers**
Alt-GoF #3: Use of alternative, virus-free vaccine platforms • Recombinant vaccines, DNA-based vaccines	• Avoid use of vaccine strains with enhanced yield relative to wild type viruses • Additional benefits discussed further below	• Only one recombinant flu vaccine is currently FDA-approved (Flublok) o Use limited to people 18 years and older o Represented less than 0.1% of vaccine distributed during 2014 – 2015 flu season	• Development and registration of new influenza vaccines is a lengthy and expensive process (8 – 10 years and 0.3 – 1 billion dollars)
** Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental Information).*			

Using viruses with wild type growth properties in lieu of high-yield vaccine viruses generated using GoF approaches would have adverse consequences for vaccine availability. Figure 15.2 illustrates three different scenarios associated with the use of wild type viruses for egg- and cell-based production of influenza vaccines, which would impact several stages of the vaccine production process. Specifically, use of wild type viruses in lieu of high-yield vaccine viruses would:[1351]

- Eliminate the need for CVV development and CVV testing, shortening the vaccine production timeline by approximately nine weeks,
- But would reduce the rate of bulk antigen production (i.e., by 12-fold, on average), and
- Minimally affect the time needed for seed strain development,[1352] potency reagent development, vaccine formulation, or lot testing/release.

Most influenza viruses grow poorly in eggs and cells. If manufacturers attempted to use strains with poor growth properties for large-scale infection of eggs/cells, the quantity of virus produced would likely be low enough, relative to egg/cellular proteins, that existing manufacturing processes would fail to produce "purified" antigen that meets FDA purity standards. This manufacturing failure would result in **no vaccine produced** (Figure 15.2, scenario 3). At best, manufacturers could pause production and attempt to adjust their purification protocols, which would extend an already lengthy production process.[1353]

Alternatively, a field isolate with exceptional growth properties that permits production of bulk antigen at reduced rates could be used to produce the same number of doses currently produced over an extended period of time (Figure 15.2, scenario 1) or to produce a smaller number of doses on the current production timescale (Figure 15.2, scenario 2). (It should be noted that influenza vaccine production experts deemed this scenario – field isolates with unusually high yields and correct antigenic properties – highly unlikely.)[1354] To illustrate the consequences for vaccine availability in scenarios 1 and 2, there is an assumption that the yields of the exceptional field isolate are approximately one-third those of a typical seasonal H1N1 strain.[1355,1356,1357] Use of such an isolate to produce the same number of doses would lengthen the time needed for bulk antigen production by three-fold, from 19 weeks to 57 weeks, which, coupled with six weeks for seed strain development, would result in release of vaccine **63 weeks** after initiation of manufacturing. During the 2009 H1N1 pandemic, this vaccine would not have been available until April 2015, well after peak waves of flu activity and near the end of the pandemic

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1351 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1352 Industry representatives noted that whether a high-growth reassortant or a field isolate were used for large-scale production, some degree of passaging by manufacturers is required for optimizing infection conditions using the particular strain and for preparing enough seed virus for large-scale infection of eggs/cells.

1353 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1354 Ibid.

1355 During the 2009 H1N1 influenza pandemic, the yields of the initial H1N1 pdm high-growth reassortant (HGR) strain were approximately one-third those of a typical seasonal H1N1 HGR. This strain was used to produce clinical lot material, thus demonstrating that this yield reduction does not preclude preparation of sufficiently pure antigen. However, subsequently, the initial HGR was extensively passaged to increase its yield to enable preparation of sufficient quantities of vaccine in a timely manner.

1356 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1357 WHO. Availability of a new candidate reassortant vaccine virus for pandemic (H1N1) 2009 virus vaccine development http://www.who.int/csr/disease/swineflu/guidance/vaccines/candidates/ep122_2009_0608_availability_of_new_cr_vaccine_virus_nibrg-121-final.pdf?ua=1. Last Update Accessed September 15, 2015.

period.[1358,1359,1360,1361] In the context of seasonal influenza vaccine production, this production timeline would necessitate strain selection more than one year in advance of the start of the target flu season. Given the challenges for such long-term predictions of the dominant circulating strains and the likelihood of antigenic drift over the course of the production year, the vaccine strains would be highly unlikely to match the circulating strains during the target flu season, leading to reduced vaccine efficacy.[1362] Alternatively, the exceptional field isolate could be used to produce a smaller number of doses on the standard production timescale (scenario 2). During a pandemic, this shortcoming would translate to a **two-fold** reduction in vaccine availability, while use of such a field isolate for seasonal flu vaccine production would result in production of **one-third** the typical number of doses (i.e., enough doses to vaccinate just under 20% of the US population).[1363,1364] Furthermore, in either scenario, the choice of a vaccine strain would be guided by the growth properties of strains of interest, which may lead to the production of vaccines that poorly match the antigenicity of the dominant circulating strain.[1365] Finally, it is noted that use of an attenuated field isolate would add nine weeks to the timelines described above, for development and testing of the attenuated reassortant, further delaying release of the vaccine and/or reducing the number of doses produced.

1358 The US Public Health Emergency for H1N1 influenza expired on June 23, 2010, and the CDC's official estimates for pandemic H1N1-associated morbidity and mortality in the US span April, 12 2009 through April 10, 2010.

1359 CDC. 2009 H1N1 Flu. http://www.cdc.gov/h1n1flu/. Last Update Accessed September 15, 2015.

1360 Shrestha SS *et al* (2011) Estimating the burden of 2009 pandemic influenza A (H1N1) in the United States (April 2009-April 2010). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 52 Suppl 1: S75-82

1361 Borse RH *et al* (2013) Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. *Emerging infectious diseases* 19: 439-448

1362 (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

1363 Current vaccine production processes lead to vaccine release at approximately week 37 following emergence of a new pandemic strain. Using a field isolate, this timeline would comprise strain isolation (2 weeks), vaccine seed strain development (6 weeks), and large-scale vaccine production (29 weeks). Given production at one-third of the typical rate, this would result in approximately half of the number of doses produced relative to use of a standard strain over a 19-week production period.

1364 Because CVVs for seasonal influenza strains are produced in advance of strain selection, using field isolates in lieu of CVVs would not alter the basic components of the industrial production process. Thus, use of a field isolate with virus yields approximately one-third those of a typical high-growth reassortant would lead to the production of approximately one-third the typical amount of vaccine over the course of the same time period. As approximately 170 million doses of influenza vaccine are produced annually, this would result in production of about 55 million doses, or enough to vaccinate 18% of the US population.

1365 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

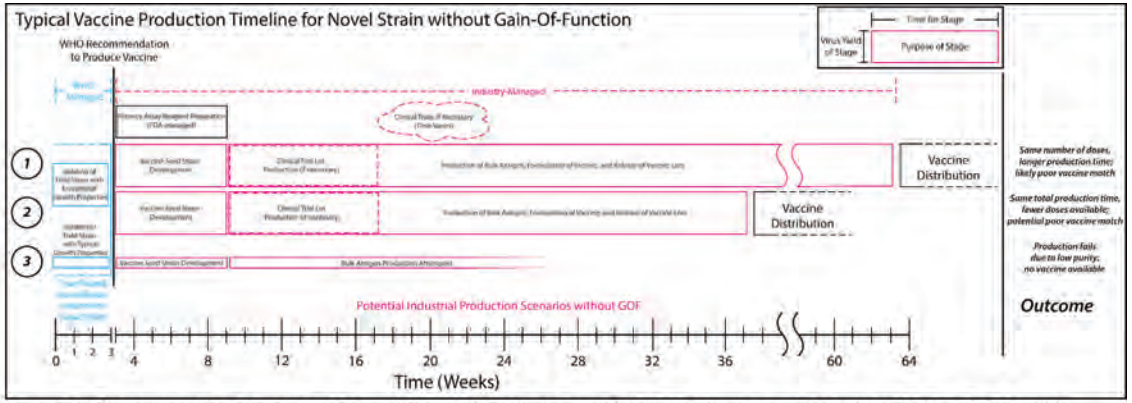

Figure 15.2. Consequences for influenza vaccine production timelines if strains with field-like growth properties were used in lieu of high-growth reassortants generated through GoF approaches. In Scenario 3, the growth properties of the field strain are so low that virus antigen cannot be purified to FDA standards; thus, no vaccine is produced. In Scenarios 1 and 2, it is assumed that a field isolate with exceptional growth properties (4-fold greater than average, leading to production of bulk antigen at approximately one-third the average rate), is used. This strain could be used to produce the same number of doses over a greater period of time (more than one year, Scenario 1) or could be used to produce a smaller number of doses in the same period of time (two- to three-fold fewer doses, Scenario 2). In either Scenarios 1 or 2, manufacturers are likely to prioritize growth properties of the strain over antigenicity, leading to potentially poor vaccine match and reduced vaccine efficacy.

Additionally, neither alternative (i.e., use of wild type strains or use of novel reassortants with wild type growth properties) can be implemented immediately. Large-scale production of field isolates for the purpose of producing inactivated vaccines would pose significant risks to vaccine manufacturers prior to the inactivation step, presumably requiring the construction of new manufacturing facilities capable of virus production under higher biocontainment conditions. Of note, field isolates cannot be used as a basis for live vaccines due to their pathogenicity. The alternative, use of attenuated vaccine viruses with wild type growth properties, would necessitate the development, and perhaps subsequent FDA licensing, of novel vaccine backbone strains that attenuate but do not confer high growth to reassortant viruses.

As described above, production of virus-based vaccines in eggs/cells necessitates passaging of the antigenic strain of interest to produce enough stock virus to infect eggs/cells for large-scale manufacturing, which inevitably selects for higher-yield viruses due to the high mutation rate of influenza viruses.[1366] If this passaging were considered to be a GoF approach, in addition to the approaches described above that deliberately enhance the yields of vaccine viruses, then completely avoiding manipulations that are reasonably expected to enhance virus production precludes production of egg- and cell-based influenza vaccines. In that case, virus-free vaccine platforms, such as recombinant or DNA-based vaccines, represent an alternative to egg- and cell-based flu vaccines (Table 15.9).[1367,1368,1369] However, the one recombinant flu vaccine that is commercially available is only approved for use in people 18 years of age and older and represented just 50,000 of more than 140 million doses administered during the 2014 – 2015 flu season.[1370,1371] Although other recombinant vaccines are in late stages of development, given the long and expensive product development cycle for new influenza vaccines – spanning eight to 12 years and costing 300 million to one billion dollars including research, clinical development, and registration with the FDA – alternative, virus-free flu vaccine platforms are not a viable *replacement* for egg- and cell-based vaccines in the immediate future.[1372]

15.2.3.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches for Current Influenza Vaccine Production

The strengths and limitations of GoF and alt-GoF approaches that could be used for the current production of influenza vaccines are summarized in Table 15.9. Taken together, this analysis demonstrates that GoF approaches to enhance the growth of attenuated vaccine strains are a **uniquely critical component** of the current ability to produce sufficient and effective vaccines for seasonal and pandemic influenza. The use of field strains or of novel reassortant strains with field-like growth properties for egg- and cell-based vaccine production would have adverse consequences for the availability and efficacy of vaccines, including the possibility that no vaccine could be produced, and neither approach could be implemented immediately. Recombinant vaccines and other virus-free vaccine platforms represent a promising approach for future influenza vaccine production, but the one recombinant vaccine that is currently licensed represents less than 1% of seasonal influenza vaccines administered annually, and lengthy regulatory processes will delay the availability of additional virus-free vaccines in the future.

1366 Parvin JD *et al* (1986b) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. *Journal of virology* 59: 377-383

1367 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

1368 Kim JH, Jacob J (2009) DNA vaccines against influenza viruses. *Current topics in microbiology and immunology* 333: 197-210

1369 Bright R. Review of New Vaccine Platforms and Influenza Vaccine Pipeline. http://www.who.int/influenza_vaccines_plan/resources/bright.pdf. Last Update Accessed September 15, 2015.

1370 Dowling B. Protein Sciences' N.Y. Factory Licensed For Flu Vaccine Production. http://www.courant.com/business/hc-protein-sciences-pearl-river-approval-20150513-story.html. Last Update 13 May 2015. Accessed 14 September 2015.

1371 Protein Sciences. Flublok. http://www.proteinsciences.com/FVAC.htm. Last Update Accessed September 15, 2015.

1372 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

15.2.4 Benefits of GoF Research that Enhances Production of Influenza Viruses to Scientific Knowledge and to Future Influenza Vaccine Production Practices

In this section of the report, the benefits of GoF research that enhances virus production to scientific knowledge and to vaccine production in the future are evaluated. As noted above, academic research in this phenotypic category is focused on enhancing the yields of vaccine viruses and has a clear translational focus, on generating higher-yield vaccine strains that can be used for vaccine production, generating information about high-yield markers that can be incorporated into vaccine strains, and/or deepening understanding of the mechanisms regulating the growth of vaccine viruses to provide a foundation for the development of higher-yield vaccine strains in the future. To provide context for these experimental goals, first, shortcomings in the current system for production of influenza vaccines are reviewed. Next, the potential benefits of GoF research to scientific knowledge about the genetic and phenotypic traits underlying high-growth of influenza viruses in eggs and cells, relative to alternative experimental approaches are evaluated. Finally, the section concludes with evaluation of how the insights and products arising from GoF research may be applied to vaccine production to further improve existing production practices and benefit public health in the future.

15.2.4.1 Shortcomings of Current Systems for Production of Influenza Vaccines

Interviews with stakeholders in the influenza research and public health communities highlighted that the lengthy production timelines for existing egg- and cell-based vaccines critically limit the mitigating impact of influenza vaccination on the morbidity and mortality associated with influenza outbreaks. In the context of seasonal flu epidemics, existing production timelines necessitate strain selection nine months in advance of the peak of the target flu season.[1373] As a result, one or more vaccine strains are often imperfectly matched to circulating strains, either due to poor strain selection (i.e., incorrect prediction of which strain would predominate in nature) or antigenic drift of the selected strain in nature during the course of vaccine production, which reduces the efficacy of the vaccine.[1374] In the context of pandemics, vaccines are simply unavailable to protect the public until at least six months into the outbreak.[1375,1376] Additionally, CVVs may acquire mutations that alter their antigenicity during growth in eggs or in cells, a third shortcoming that results in poor vaccine match and that can affect the production of seasonal and pandemic vaccines. In particular, H3N2 strains often acquire antigenicity-altering mutations upon growth in eggs, which is especially concerning given that H3N2 strains tend to cause more severe disease than H1N1 strains.[1377,1378,1379,1380]

The yields of vaccine viruses establish the rate of bulk antigen production and thus serve as a key determinant of the time needed for vaccine production. Some strains, including H3N2 strains and many

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[1373] Ibid.

[1374] (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

[1375] Borse RH *et al* (2013) Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. *Emerging infectious diseases* 19: 439-448

[1376] (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

[1377] (2015v) Candidate vaccine virus development. Interviews with Influenza Researchers Involved in Candidate Vaccine Virus Development.

[1378] Barman S *et al* (2015) Egg-adaptive mutations in H3N2v vaccine virus enhance egg-based production without loss of antigenicity or immunogenicity. *Vaccine* 33: 3186-3192

[1379] Huang SSH *et al* (2011) Comparative Analyses of Pandemic H1N1 and Seasonal H1N1, H3N2, and Influenza B Infections Depict Distinct Clinical Pictures in Ferrets. *PLoS ONE* 6: e27512

[1380] Kaji M *et al* (2003) Differences in clinical features between influenza A H1N1, A H3N2, and B in adult patients. *Respirology (Carlton, Vic)* 8: 231-233

zoonotic influenza strains, routinely produce low-yield CVVs, and any strain may unexpectedly produce a poorly growing CVV.[1381,1382] For example, the 2009 H1N1 pandemic CVV exhibited production yields approximately one-third those of a typical H1N1 seasonal CVV.[1383] In either case, the need to extensively passage a low-yield CVV to render it suitable for large-scale production, as happened in 2009, and/or to utilize a sub-par CVV for production, delays manufacturing and subsequent release of the vaccine.[1384,1385] Additionally, even high-growth CVVs typically exhibit reduced yields relative to vaccine backbone strains, indicating that CVV yields could be further increased. Thus, the limited production yields of CVVs represent a gap that compromises the efficacy and utility of existing influenza vaccines by lengthening egg- and cell-based vaccine production timelines. Furthermore, the fact that existing strategies for CVV development do not consistently produce high-yield strains highlights the incomplete understanding of the genetic determinants underlying high growth in eggs and cells.

15.2.4.2 Benefits of GoF Research That Enhances Virus Production to Scientific Knowledge

15.2.4.2.1 Benefits and Limitations of GoF Approaches

Several GoF approaches can be used to discover mutations associated with high growth of vaccine backbone strains and CVVs. Serial passaging of viruses in eggs or cells is a classic method for identifying mutations that confer enhanced growth, while forward genetic screens, which involve randomly mutagenizing strains and subsequent passaging of mutant libraries to select for high-growth variants, represent a modern approach for discovery of genetic markers associated with high growth. Both approaches enable the discovery of mutations that are *sufficient* to confer higher-than-wild type levels of growth to any virus strain of interest. However, both approaches are limited by their narrow breadth; that is, the mutations that are identified may confer high growth to the studied strain only.

Comparing the sequences of CVVs with varied growth properties is another GoF method that can be used to identify mutations that are *associated* with high growth. (It should be noted that this method is considered a GoF approach because CVVs exhibit enhanced replication relative to vaccine backbone strains, as described above.) However, unlike serial passaging and forward genetics approaches, comparative sequence analysis is unlikely to uncover genetic markers associated with greater-than-wild type levels of growth because it is limited to analysis of existing isolates.

In either case, the phenotypic consequences of mutations can then be confirmed through targeted mutagenesis of the parental strain. Collectively, these approaches enable the identification of genetic traits that are *necessary* and *sufficient* to confer higher-than-wild type levels of growth to vaccine viruses, for any strain of interest. This information provides a foundation for follow-up structural, biochemical, and cell biological assays investigating the phenotypic consequences of the mutations, in order to gain insight into the mechanisms underlying the enhanced growth phenotype. Subsequently, using targeted mutagenesis to determine the effect of the marker on virus growth in a new strain context provides insight into whether the marker is likely to be broadly useful for improving CVV yields as well as whether the phenotypic traits underlying high growth are conserved across strains.

1381 (2015y) Candidate vaccine virus development. Interviews with Influenza Researchers Involved in Candidate Vaccine Virus Development.

1382 Barman S *et al* (2015) Egg-adaptive mutations in H3N2v vaccine virus enhance egg-based production without loss of antigenicity or immunogenicity. *Vaccine* 33: 3186-3192

1383 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1384 Ibid.

1385 WHO. Availability of a new candidate reassortant vaccine virus for pandemic (H1N1) 2009 virus vaccine development http://www.who.int/csr/disease/swineflu/guidance/vaccines/candidates/ep122_2009_0608_availability_of_new_cr_vaccine_virus_nibrg-121-final.pdf?ua=1. Last Update Accessed September 15, 2015.

15.2.4.2.2 Benefits and Limitations of Alt-GoF Approaches

Alternative experimental approaches ("alt-GoF") can also be used to uncover genetic markers *associated* with high growth. Sequence comparison of wildtype strains with varied growth properties may provide insight into mutations that confer a growth advantage. Of note, because of the importance of genetic context on multi-genic traits such as fitness, mutations that confer high growth to wildtype strains may not confer high growth to vaccine strains (i.e., reassortants that include the HA and NA from the field isolate and the remaining six genes from a vaccine backbone strain). Similar to comparative sequence analysis of CVVs, this approach depends on the existence of high-growth strains in nature and cannot identify mutations that confer exceptional yields.

Genetic screens to identify mutations that reduce growth (i.e., Loss of Function, or LoF) can lead to the discovery of mutations that are *necessary* for growth. A major limitation of this approach is that it may uncover mutations that reduce growth for "trivial," reasons, i.e., that modulate critical aspects of virus function that are necessary for viability but do not directly contribute to high growth. An additional drawback is that it is much less efficient than its GoF counterpart because mutants must be screened for reduced growth (versus selection for high growth through passaging). Finally, the utility of the information gleaned from LoF screens also depends on the existence of high-growth strains in nature.

LoF approaches may also be used to confirm that a particular amino acid residue (discovered through GoF or alt-GoF approaches) is necessary for high growth. However, the marker may not be sufficient to enhance growth if introduced into a different strain, limiting the utility of this result for vaccine production.

15.2.4.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches for Scientific Knowledge

The scientific knowledge benefits and limitations of all GoF and alt-GoF approaches discussed in this section, with respect to the ability of each approach to provide insight into the mechanisms underlying the growth of influenza vaccine viruses, are summarized in Table 15.10. GoF approaches are **uniquely capable** of discovering mutations that enhance the growth of any vaccine virus strain to greater-than-wildtype levels. In addition, GoF approaches are **uniquely capable** of demonstrating that particular mutations are necessary and sufficient to enhance the growth of vaccine viruses. Together, this information provides a strong foundation for follow-up studies investigating the mechanistic basis of high growth of vaccine viruses.

Alternative approaches have significant limitations for the study of mechanisms governing the growth of vaccine viruses. Comparative sequence analysis of wild type isolates is limited to the study of phenotypes underlying naturally high levels of growth, and the information gleaned from these studies may not translate to vaccine viruses. LoF approaches are inefficient, and genetic markers that are necessary for high growth may not be sufficient to enhance growth if introduced into a different strain.

Furthermore, GoF approaches to confirm that particular markers confer high growth are **uniquely critical** for generating information that can be translated to the vaccine production process. The phenotypic consequences of incorporating mutations that are associated with high growth or that are necessary for high growth into vaccine viruses are too uncertain to be applied to vaccine production.

Table 15.10. Summary of the Benefits of GoF Approaches That Enhance Virus Production

Scientific Knowledge Benefits – What Is the Mechanistic Basis of High Growth of Influenza Viruses in Eggs and Cells?

Experimental Approach	Benefits	Limitations
GoF #1 [5]*: Serial passaging of viruses in eggs or cells **GoF #2 [6]**: Forward genetic screen to identify mutations sufficient to confer increased virus production on virus backbones.	• Identify new genetic traits that are **sufficient** to enhance the growth of any virus to greater than wild type levels	• Associative – whether mutations are necessary to enhance growth must be experimentally confirmed • Narrow breadth – results may not generalize to other influenza strains
GoF #3 [7]: Comparative sequence analysis of CVVs with varied growth properties to identify genetic traits associated with high growth	• Identify genetic traits that are associated with naturally high levels of growth of existing CVVs	• Associative - whether mutations are necessary and sufficient to enhance growth must be experimentally confirmed • Utility depends on the availability of CVVs with varied growth properties
GoF #4 [8,9]: Targeted genetic modification of parental virus to introduce mutations shown to be associated with enhanced growth • *Confirm* the phenotypic effects of a particular mutation in a known strain or *validate* its phenotypic effects in a new strain context	• Identify genetic traits that are **necessary and sufficient** to enhance the growth of any virus	• Narrow breadth - results may not generalize to other influenza strains
Alt-GoF #1 [1]: Comparative sequence analysis of wild type strains with varied growth properties to identify genetic traits associated with high growth	• Identify genetic traits that are associated with naturally high levels of growth of existing field strains	• Associative - whether mutations are necessary and sufficient to enhance growth must be experimentally confirmed • Utility depends on the availability of field isolates with varied growth properties • Epistasis – mutations that confer high growth to wild type strains may not be conserved in vaccine strains
Alt-GoF #2 [2,3]: Loss of Function approaches • Forward genetic screen to identify new mutations that attenuate virus production • Targeted genetic modification of parental virus to mutate amino acid residues associated with high growth	• Identify genetic traits that are necessary for naturally high growth of existing field strains	• Genetic markers may not be sufficient to enhance growth in a different strain context • Inefficient – screening for attenuated growth is less efficient than selecting for enhanced growth • Narrow breadth - results may not generalize to other influenza strains

Table 15.10. Summary of the Benefits of GoF Approaches That Enhance Virus Production		
Scientific Knowledge Benefits – What Is the Mechanistic Basis of High Growth of Influenza Viruses in Eggs and Cells?		
Experimental Approach	**Benefits**	**Limitations**
** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify experimental approaches described in the landscape tables (Supplemental Information).*		

15.2.4.3 Public Health Benefits of GoF Research that Enhances Virus Production

GoF approaches that enhance virus production have the potential to improve existing vaccine production practices by addressing two shortcomings in the current process for egg- and cell-based vaccine production: (1) some strains acquire mutations that alter antigenicity during growth in eggs or cells, leading to poor vaccine match, and (2) production timelines are too long. As described above, the yield of CVVs governs the rate of bulk antigen production in eggs/cells and thus serves as a key determinant of the length of time needed for egg- and cell-based vaccine production. Lengthy vaccine production timelines impact the quality and availability of seasonal and pandemic flu vaccines differently. In the context of seasonal flu epidemics, existing production timelines necessitate strain selection nine months in advance of the peak of the target flu season.[1386] As a result, one or more vaccine strains are often imperfectly matched to circulating strains, which reduces the efficacy of the vaccine.[1387] In the context of pandemics, vaccines are simply unavailable to protect the public until at least six months into the outbreak.[1388] (It is noted that the impact of improving vaccine availability and efficacy during influenza pandemics and seasonal epidemics will be further explored using quantitative methods in the quantitative benefit assessment section of this report (Section 9.12).)

This sub-section first evaluates how GoF research may benefit the availability and efficacy of vaccines by generating genetically stable, high-yield CVVs. Then, alternative approaches that have potential to similarly benefit vaccine production by shortening vaccine production timelines are evaluated. Finally, alternative scientific and technical innovations that may improve the quality and availability of vaccines through completely different mechanisms are analyzed.

15.2.4.3.1 Benefits of GoF Approaches to Future Influenza Vaccine Production

GoF research that generates genetically stable, higher-yield CVVs can be translated to vaccine production through direct use of lab-generated CVVs or through incorporation of genetic markers that confer high-growth into existing CVVs using targeted mutagenesis. Of note, studies that increase the yields of vaccine backbone viruses generate more broadly applicable information than those focusing on particular CVVs.

As described above, this research can address two shortcomings in the current vaccine production process. First, information about genetic markers that confer high growth without altering antigenicity can benefit the production of vaccines for strains that readily mutate during passage in eggs or cells, such as H3N2 strains. Specifically, the use of new, GoF-derived genetically stable CVVs would enable the production of vaccines that match the antigenicity of the selected strains, which translates to improved vaccine efficacy. Second, the use of higher-yield vaccine viruses or the incorporation of high-growth markers into existing CVVs can benefit the production of vaccines for any strain by increasing the rate of bulk antigen production and thereby shortening vaccine production timelines.

One key constraint on the benefits afforded by improvements to CVV yields is the limited production capacity of eggs and cells. Current *egg*-based vaccine production systems are at or near maximal levels of production, suggesting that the benefits of GoF research are largely limited to improving the growth of "poor" CVVs.[1389] However, because many CVVs based on zoonotic viruses and seasonal H3N2 viruses

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[1386] Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

[1387] (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

[1388] Borse RH *et al* (2013) Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. *Emerging infectious diseases* 19: 439-448

[1389] (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

grow poorly in eggs, simply improving their production would significantly benefit public health.[1390,1391] In contrast, the production capacities of *cell*-based systems have not yet plateaued, thus GoF research that improves CVV yields has the potential to benefit production of vaccines for all influenza sub-types using cell-based systems.[1392]

Importantly, because these minor modifications to existing CVVs are not likely require FDA approval for use in vaccine production, these benefits can be realized in the immediate future.[1393]

15.2.4.3.2 Benefits of Alternative Approaches with Potential to Shorten Vaccine Production Timelines

Several alternative approaches have potential to improve the availability and efficacy of vaccines by shortening vaccine production timelines through different mechanisms. First, an alternative approach for improving vaccine virus yields without enhancing the inherent growth properties of CVVs is through modulation of the host cells that are used to produce virus. Specifically, identification of host genes that suppress viral growth provides a basis for development of specialized knockout cell lines that permit higher virus yields.[1394] The key drawbacks to this approach are that research on whether such cell lines will support high growth of a wide variety of influenza strains is limited, and currently, only one cell-based vaccine that could potentially make use of this technology is licensed in the US.[1395] Furthermore, cell lines must undergo extensive testing in order to be FDA-approved for influenza vaccine production prior to their commercial use, which will delay realization of this benefit.[1396,1397] Finally, the risk associated with GoF experiments that enhance virus production inheres in the fact that researchers are working with increased viral titers relative to experiments using wildtype strains should be noted. As the host cell modulation approach leads to the same consequence – i.e., that researchers handle higher quantities of virus – this alt-GoF approach does not reduce risk relative to GoF approaches that enhance viral titer through modulation of the virus.

An adjuvant is a substance that is added to a vaccine to boost the body's immune response to the vaccine, and including an adjuvant in a vaccine may enable the use of a smaller quantity of antigen to induce the same level of protection ("dose sparing").[1398] Thus, incorporating adjuvants into existing egg- and cell-based vaccines represents a different strategy for shortening production timelines, by enabling production of the same number of doses over a shorter period of time. Most licensed vaccines in the US are not adjuvanted – one seasonal vaccine containing adjuvants was recently approved for use in people aged 65

1390 (2015y) Candidate vaccine virus development. Interviews with Influenza Researchers Involved in Candidate Vaccine Virus Development.

1391 (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

1392 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1393 Ibid.

1394 Hamamoto I *et al* (2013) High yield production of influenza virus in Madin Darby canine kidney (MDCK) cells with stable knockdown of IRF7. *PloS one* 8: e59892

1395 TABLE. Influenza vaccines — United States, 2015–16 influenza season. http://www.cdc.gov/flu/protect/vaccine/vaccines.htm. Last Update Accessed September 14, 2015.

1396 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

1397 FDA. Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications http://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm202439.pdf. Last Update Accessed September 15, 2015.

1398 CDC. Vaccine Adjuvants. http://www.cdc.gov/vaccinesafety/concerns/adjuvants.html. Last Update Accessed September 15, 2015.

and older, and one licensed pandemic influenza vaccine contains adjuvants.[1399,1400,1401,1402] Nonetheless, use of adjuvants to improve the immunogenicity of seasonal influenza vaccines is an active area of research. The major barrier to realization of this benefit is that existing vaccines that are re-formulated with adjuvant are considered new drugs by the FDA and as such must undergo the standard licensure pathway for unadjuvanted vaccines.[1403,1404,1405] Although new seasonal inactivated influenza vaccines may be considered for the accelerated regulatory pathway, which requires less extensive clinical trials than the traditional regulatory pathway (coupled with industry commitment to post-licensure studies), even the accelerated pathway spans over five years.[1406,1407]

Developing new vaccine platforms with faster production timelines represents a third alternative approach for shortening the time needed for production of strain-specific vaccines. Recombinant vaccines, which are virus-free vaccines comprised of recombinant influenza proteins produced in insect cells or other protein expression systems such as plants, represent the most developed and promising approach.[1408,1409] The major benefit of recombinant vaccines is that production can be rapidly scaled up in response to the emergence of a novel pandemic strain, leading to production of clinical trial material one to two months sooner than egg- and cell-based production systems and commercial release of vaccine six to eight weeks sooner than traditional platforms.[1410] Although only one recombinant vaccine is currently FDA-licensed, several other recombinant vaccines are in late stages of development, and experts in the influenza vaccine field expect the production and use of this type of vaccine to increase over the next several decades.[1411,1412] However, as mentioned above, the time needed for completion of clinical trials and

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1399 Ibid.

1400 Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted. http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm376289.htm. Last Update Accessed September 15, 2015.

1401 FDA. FDA approves first seasonal influenza vaccine containing an adjuvant. FDA News Release. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm474295.htm. Last Update November 24, 2015. Accessed November 28, 2015.

1402 Novartis. FLUAD® (MF59®-Adjuvanted Influenza Vaccine) Fact Sheet. https://www.novartis.com/sites/www.novartis.com/files/Fluad_Fact_Sheet.pdf. Last Update Accessed September 15, 2015.

1403 Montomoli E *et al* (2011) Current adjuvants and new perspectives in vaccine formulation. *Expert Rev Vaccines* 10: 1053-1061.

1404 Food and Drug Administration. Vaccine Product Approval Process. http://www.fda.gov/BiologicsBloodVaccines/DevelopmentApprovalProcess/BiologicsLicenseApplicationsBLAProcess/ucm133096.htm. Last Update 24 August 2015. Accessed 14 September 2015.

1405 Gruber M. Regulatory Pathways Supporting Development and Approval of Vaccines Formulated with Novel Adjuvant: Regulatory Considerations and Challenges. http://www.fda.gov/downloads/EmergencyPreparedness/MedicalCountermeasures/UCM292045.pdf. Last Update 2012. Accessed 14 September 2015.

1406 Food and Drug Administration. Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074794.htm. Last Update 31 May 2007. Accessed 15 September 2015.

1407 Novartis Vaccines and Diagnostics. FDA Advisory Committee Briefing Document: Fluad Seasonal Adjuvanted Trivalent Influenza Vaccine (aTIV). http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM461917.pdf. Last Update 15 September 2015. Accessed 21 September 2015.

1408 Bright R. Review of New Vaccine Platforms and Influenza Vaccine Pipeline. http://www.who.int/influenza_vaccines_plan/resources/bright.pdf. Last Update Accessed September 15, 2015.

1409 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1410 Ibid.

1411 TABLE. Influenza vaccines — United States, 2015–16 influenza season. http://www.cdc.gov/flu/protect/vaccine/vaccines.htm. Last Update Accessed September 14, 2015.

1412 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

licensing delays the ability of this technology to impact influenza vaccination systems in the US in the near term (i.e., within the next few years). Additionally, unless WHO strain selection meetings are delayed to match the shorter production timescales of alternative platforms, these seasonal recombinant vaccines will be subject to the same limitations due to strain selection and antigenic drift as egg/cell-based vaccines (though are able to adjust production mid-stream if necessary, unlike egg/cell-based systems).[1413]

The benefits and limitations of GoF and alt-GoF approaches that shorten vaccine production timelines by reducing the time needed for bulk antigen production are summarized in Table 15.11. It should be noted that several other steps of the vaccine production process are time-consuming, such as preparation of potency reagents for standardization of vaccine antigen and clinical trials (for pandemic vaccines). As bulk antigen production times shrink, these other steps may become rate-limiting, unless new methods for quantification of recombinant antigen are developed and FDA-approved.[1414]

[1413] Ibid.
[1414] Ibid.

Table 15.11. Summary of the Benefits of GoF Approaches That Enhance Virus Production

Vaccine Development Benefits – Potential Benefits of Innovations that May Shorten Production Timelines for Strain-Specific Vaccines

Scientific/technical innovation	Benefit	Limitations	Barriers
GoF: Improve yields of CVVs used for production of egg- and cell-based vaccines	Shorten production timelines for egg- and cell-based vaccines by increasing rates of bulk antigen production	• Gains for high-yield CVVs are limited by the production capacities of egg and cell systems o Egg-based production systems are already near maximum levels of productivity • Several stages of egg/cell-based production are time-consuming and may become rate-limiting	• Likely none – minor modifications to existing CVVs are unlikely to require FDA approval for use in vaccine production
Alt-GoF #1: Develop new host cell lines that permit higher levels of virus replication.	Shorten production timelines for egg- and cell-based vaccines by increasing rates of bulk antigen production	• Gains for high-yield CVVs are limited by the production capacities of egg and cell systems o Egg-based production systems are already near maximum levels of productivity • Several stages of egg/cell-based production are time-consuming and may become rate-limiting	• New cell lines must be FDA-licensed prior to their commercial use.
Alt-GoF #2: Use of adjuvants for antigen sparing	Enable production of the same number of vaccine doses in a shorter time period	• Only one adjuvanted seasonal vaccine (approved for use in adults aged 65 and older) and one adjuvanted pandemic vaccine are FDA-licensed • Several stages of egg/cell-based production are time-consuming and may become rate-limiting	• Adjuvanted vaccines are considered "new" and must be FDA-licensed prior to commercial release o Development and licensing of new vaccines is a lengthy and expensive process (requires clinical trials) o More than five years, even using accelerated regulatory pathway

Table 15.11. Summary of the Benefits of GoF Approaches That Enhance Virus Production			
Vaccine Development Benefits – Potential Benefits of Innovations that May Shorten Production Timelines for Strain-Specific Vaccines			
Scientific/technical innovation	**Benefit**	**Limitations**	**Barriers**

Alt-GoF #3: Develop new vaccine platforms with faster production timelines, such as recombinant flu vaccines	Shorten production timelines for strain-specific vaccines	• Only one alternative vaccine (Flublok, a recombinant vaccine) is FDA-licensed
		  ○ Others are in late stages of development
		• Preparation of potency reagents for standardization of vaccine antigen is time-consuming and may become rate-limiting
		  ○ Alternative standardization assays could be used
			• Development and licensure of new influenza vaccines is a lengthy and expensive process
			• Alternative standardization assays that do not depend on FDA-generated potency reagents must be FDA-licensed
			• Will be subject to limitations associated with strain selection far in advance of flu season unless WHO strain selection meetings are delayed to match shorter production timescales

15.2.4.3.3 Benefits of Alternative Approaches with Potential to Improve the Availability of Pandemic Influenza Vaccines through Different Mechanisms

Because each of the GoF and alt-GoF approaches described above involves initiation of manufacturing following the start of the pandemic, none can address the gap in protection in the immediate aftermath of emergence of a novel strain. Several alternative approaches aim to proactively protect the public against influenza pandemics, namely development of universal vaccines and development and stockpiling of pre-pandemic vaccines.

A universal or broad-spectrum flu vaccine would obviate the need for production of a strain-specific vaccine in response to the emergence of a novel pandemic strain. Such a vaccine could be administered in advance of a pandemic, generating pre-existing immunity in the population, or could be stockpiled and immediately deployed following the start of a pandemic. However, development of a universal or broad-spectrum vaccine represents a scientifically challenging prospect. Although multiple research efforts are underway, influenza and vaccinology experts disagree about whether a universal flu vaccine is achievable, and one expert felt that a ten to 20 year time frame for development of a universal vaccine is optimistic.[1413,1416,1417]

Development of pre-pandemic vaccines against circulating zoonotic influenza strains with pandemic potential would also lead to faster vaccine availability during a pandemic caused by a closely related strain. Developing pre-pandemic CVVs and carrying out clinical trials would shorten vaccine production timelines, and stockpiling bulk antigen would allow for near-immediate deployment of vaccine following emergence of a pandemic strain. In addition, manufacturers' experience with production of the vaccine would likely streamline subsequent large-scale production during the pandemic.[1418] Although the pre-pandemic vaccine strain is unlikely to exactly match the strain that emerges to cause a pandemic, use of adjuvants and prime-boost regimens broaden the protection that can be achieved using a strain-specific vaccine, such that pre-pandemic vaccines are highly likely to provide some level of protection against infection with a similar strain.[1419,1420,1421,1422,1423]

The benefit of developing pre-pandemic vaccines is constrained by the fact that resources for the development and stockpiling of pre-pandemic vaccines are limited.[1424] The number of pre-pandemic CVVs that can be produced is constrained by two factors: (1) the number of facilities that can produce pre-pandemic CVVs using Good Manufacturing Processes (GMP) is limited, and (2) CVVs used for

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1413 Rudolph W, Ben Yedidia T (2011) A universal influenza vaccine: where are we in the pursuit of this "Holy Grail"? *Human vaccines* 7: 10-11

1416 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1417 (2015v) Influenza Vaccines. Interviews with Influenza Researchers.

1418 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1419 Ibid.

1420 (2015s) Influenza Vaccines. Interviews with Public Health Professionals Involved in Preventing and Responding to Influenza Outbreaks.

1421 Smith GE *et al* (2013) Development of influenza H7N9 virus like particle (VLP) vaccine: homologous A/Anhui/1/2013 (H7N9) protection and heterologous A/chicken/Jalisco/CPA1/2012 (H7N3) cross-protection in vaccinated mice challenged with H7N9 virus. *Vaccine* 31: 4305-4313

1422 Middleton D *et al* (2009) Evaluation of vaccines for H5N1 influenza virus in ferrets reveals the potential for protective single-shot immunization. *Journal of virology* 83: 7770-7778

1423 Khurana S *et al* (2010) Vaccines with MF59 adjuvant expand the antibody repertoire to target protective sites of pandemic avian H5N1 influenza virus. *Sci Transl Med* 2: 15ra15

1424 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

vaccine production must undergo extensive safety and characterization testing, which is resource-intensive.[1425] Further along in the pre-pandemic vaccine production pipeline, the expense associated with production of clinical lot material, clinical trials, and stockpiling of bulk antigen practically limits the number of pre-pandemic vaccines that can be taken to each stage of the pipeline and the quantity of bulk antigen that can be stockpiled.[1426]

The strengths and limitations of different strategies for improving the availability of pandemic influenza vaccines are summarized in Table 15.12. Taken together, this analysis demonstrates that universal vaccines are not a viable option for protection against pandemic influenza in the near future, but that development of pre-pandemic vaccines represents a promising strategy due to their ability to provide broad-spectrum protection when adjuvanted. However, because resources limit the scope of the USG's investment in pre-pandemic vaccines, these vaccines will serve to bridge the gap between the emergence of a novel strain and widespread availability of vaccines and must be complemented by innovations to shorten vaccine production timelines. Though one of several approaches that can achieve this benefit, GoF research to improve CVV yields represents the only strategy for achieving near-term benefits because it capitalizes on existing infrastructure and faces fewer regulatory barriers to translation than other approaches.

[1425] (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

[1426] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

Table 15.12. Summary of the Benefits of GoF Approaches That Enhance Virus Production			
Vaccine Development Benefits – Innovations That Can Improve the Availability of Pandemic Influenza Vaccines			
Scientific/technical innovation	**Benefit**	**Limitations**	**Barriers**
Alt-GoF: Universal or broad-spectrum influenza vaccine	Population will already have immunity against novel strains that emerge and/or can be immediately vaccinated	Universal and broad-spectrum influenza vaccines do not yet exist	Scientifically challenging – influenza experts disagree about whether development of a universal vaccine is feasible • One influenza vaccine production expert estimates that a 10 – 20 year time frame is optimistic
GoF or alt-GoF: Invest in pre-pandemic vaccine development* • CVVs, clinical trials, stockpiling of bulk antigen	Shorten production timelines • Refine vaccine formulation through clinical trials • Immediate availability of stockpiled antigen • Manufacturing experiences facilitates subsequent large-scale production	Scope of pre-pandemic vaccine development limited by availability of funds	Pre-pandemic vaccine strain unlikely to match the strain that emerges • Use of adjuvants and prime/boost regimens broaden protection
Shorten production timelines for strain-specific vaccines	**Alt-GoF**: Develop new vaccine platforms with faster production timelines	Shorter vaccine production timelines would enable earlier release of vaccine	• Only one recombinant influenza vaccine is currently FDA-approved • Other alternative vaccine platforms are in development
	Alt-GoF: Use of adjuvants for antigen sparing	Would enable production of the same number of doses in a shorter period of time, enabling earlier vaccine release	Only one seasonal and one pandemic vaccines with adjuvants are licensed
	GoF or alt-GoF: Shorten production timelines for egg- and cell-based vaccines*	Shorter vaccine production timelines would enable earlier release of vaccine	Gains for high-yield CVVs are limited by the production capacities of egg and cell systems
**Both GoF and alt-GoF approaches can inform this benefit.*			

15.2.4.3.4 Benefits of Alternative Approaches with Potential to Improve the Efficacy of Seasonal Influenza Vaccines through Different Mechanisms

Ultimately, GoF research and alternative approaches that shorten vaccine production timelines will benefit public health during seasonal flu epidemics by enabling strain selection closer to the start of the target flu season, which increases the likelihood that the vaccine strains will match circulating strains. However, with the exception of universal flu vaccines, none of the strategies described above can eliminate the need to choose vaccine strains in advance of flu season, thus vaccine mismatch remains a possibility unless other innovations are pursued in tandem. Several other approaches have potential to improve vaccine match through alternative mechanisms.

A universal or broad-spectrum vaccine would benefit public health responses to seasonal flu epidemics by obviating the need for yearly production of strain-specific vaccines, but this strategy represents a challenging, long-term approach.

As vaccine mismatch is sometimes due to incorrect prediction of which strains will predominate six to nine months hence, improving strain selection capabilities represents another approach to increasing the likelihood of vaccine match. Both GoF and alt-GoF approaches can improve strain selection capabilities, described in detail in Section 15.5.5.1 and briefly summarized here. First, both GoF and alt-GoF approaches have potential to improve the quality and quantity of the antigenic characterization data upon which strain selection decisions are based, thereby strengthening the robustness of the decision-making process. Second, GoF approaches are critical for advancing the development of methods for predicting antigenic drift, including experimental methods and computational methods. These methods would enable production of vaccines based on future, antigenically drifted strains, which will match circulating viruses at the time of vaccine deployment. Collectively, the benefits that can be achieved through both GoF and alt-GoF approaches depend on scientific advancements as well as expansion of sequencing capabilities at diagnostic labs that originally collect and characterize clinical samples. Both barriers will be challenging to overcome, though small improvements to the state of the science and to surveillance infrastructure will yield benefits. Thus the timescale for realization of these benefits is uncertain.

The strengths and limitations of different strategies for improving the efficacy of seasonal influenza vaccines are summarized in Table 15.13. Universal vaccines represent the only strategy with potential to fully "solve" the vaccine mismatch problem but are in early stages of development and represent a long-term solution at best. All other approaches hold promise for improving the likelihood of vaccine match in the near future. These approaches are complementary; that is, each approach addresses different underlying gaps in current scientific and technical capabilities that contribute to vaccine mismatch. Thus, these approaches complement each other as part of comprehensive strategy for improving the quality of seasonal influenza vaccines. This includes GoF research that improves the yields of CVVs, thus shortening vaccine production timelines, as well as GoF research that leads to the development of genetically stable CVVs that retain the antigenicity of the parental strains.

Table 15.13. Innovations That Can Address Seasonal Influenza Vaccine Gaps Associated with Long Vaccine Production Times

Gap	Scientific/technical innovation	Limitations	Barriers
Vaccine strains are often imperfectly matched to circulating strains	**Alt-GoF**: Universal or broad-spectrum influenza vaccine	Universal and broad-spectrum influenza vaccines do not yet exist	Scientifically challenging – influenza experts disagree about whether development of a universal vaccine is feasible • One influenza vaccine production expert estimates that a 10 – 20 year time frame is optimistic
	GoF: Development of genetically stable CVVs that are antigenically similar to parental strains	Limited to reducing the likelihood of vaccine mismatch for those vaccine strains that drift as a result of production	FDA approval may be required for commercial use of new CVVs
Incorrect strain selection: an unexpected strain rises to prominence in nature during the vaccine production process	**GoF or alt-GoF**: Improve strain selection capabilities:* • Improve the quality and quantity of antigenic characterization data considered during strain selection decision • Predict antigenic drift, enabling production of vaccines using drifted strains	Limited to reducing the likelihood of vaccine mismatch due to incorrect strain selection	Depends on advancements in science and improvements to influenza surveillance networks, the timescales of which are uncertain
Lengthy production times for egg- and cell-based vaccines necessitate strain selection six months in advance of flu season	• **GoF or alt-GoF**: Improve CVV yields* • **Alt-GoF**: Use of adjuvants for dose-sparing • **Alt-GoF**: Develop new, faster vaccine platforms	Cannot eliminate the need to choose vaccine strains in advance of flu season • The possibility of vaccine mismatch due to incorrect strain selection remains	Described above (Table 15.8)

**Both GoF and alt-GoF approaches can inform this benefit.*

15.2.4.3.5 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches for Future Influenza Vaccine Production

Production of Pandemic Influenza Vaccines

Both GoF approaches to improve CVV yields and alternative approaches have potential to reduce the time lag between the emergence of a novel pandemic strain in human populations and the widespread availability of a vaccine, thus reducing human morbidity and mortality during an influenza pandemic. GoF approaches to generate higher-yield CVVs and to identify mutations that enhance the growth of CVVs are **uniquely capable** of achieving this benefit in the immediate to near term because use of this information capitalizes on existing infrastructure and faces no regulatory barriers to translation. Developing new host cell lines that permit higher levels of virus replication represents an alternative approach for increasing CVV yields, but cell lines used for vaccine production must undergo extensive testing for FDA licensure and this approach is not less risky than working with viruses with enhanced yields. Although adjuvanted vaccines and virus-free vaccines have shorter production timelines than existing egg- and cell-based vaccines, the length and expense of licensure processes for new vaccines will delay their widespread availability. Universal flu vaccines are in early stages of development, and influenza and vaccinology experts disagree about the scientific feasibility of developing a universal vaccine. The development of pre-pandemic vaccines represents a promising strategy due to their ability to provide broad-spectrum protection when combined with adjuvants. However, because investments in pre-pandemic vaccines are resource-limited and strains that emerge are unlikely to exactly match the vaccine strain, this approach does not abrogate the need to produce vaccine during a pandemic but rather bridges the gap between strain emergence and widespread vaccine availability, thus complementing other strategies for shortening vaccine production timelines.

Production of Seasonal Influenza Vaccines

Both GoF approaches and alt-GoF approaches have potential to improve the match between seasonal influenza vaccines and strains that are circulating during flu season, thereby improving vaccine efficacy and decreasing human morbidity and mortality associated with seasonal flu epidemics. Because poor vaccine match arises from several different shortcomings in the current vaccine production system, this benefit can be achieved through several different mechanisms. One strategy is shortening the time needed to produce flu vaccines, which enables strain selection closer to the start of flu season. As described above, GoF approaches that improve the yields of CVVs are uniquely capable of achieving this benefit in the immediate to near term, though alternative approaches such as incorporating adjuvants into existing vaccines and developing virus-free vaccine platforms have strong potential to achieve this benefit over longer timescales.

A completely different strategy is to improve the production of strains that mutate to alter their antigenicity upon growth in eggs or cells, such as H3N2 strains, resulting in the production of vaccines that are poorly matched to the selected strains. GoF approaches are uniquely capable of generating high-yield, genetically stable CVVs that do not acquire antigenicity-altering mutations during passage in eggs or cells.

A third strategy for improving vaccine match is to improve strain selection capabilities, which would reduce the likelihood of mismatch due to incorrect predictions of which strains will be dominant during the forthcoming flu season. This benefit can be achieved by improving the quantity and quality of the antigenic characterization data upon which strain selection decisions are based, as well as by developing methods for prediction of antigenic drift, to enable developing of vaccines based on future, drifted strains that match circulating strains at the time of vaccine deployment. The former benefit depends on

strengthening influenza surveillance networks, and in particular expanding viral sequencing capabilities, and both benefits rely on scientific advancements. As a result, both the extent and timescales of these benefits are uncertain. Importantly, as these approaches address different underlying gaps in existing vaccine production systems, research in these areas has the potential to complement the benefits that can be achieved through the application of GoF research to vaccine production.

15.3 Influenza Viruses: Detailed Analysis of the Benefits of GoF Research That Enhances Mammalian Adaptation and Transmissibility

15.3.1 Overview of Influenza GoF Landscape

This assessment describes the benefits of GoF experimental approaches that are reasonably anticipated to enhance the transmissibility of influenza viruses in mammals, including approaches that enhance the fitness or infectivity of viruses in mammalian cells or in animals, respectively, as well as approaches that enhance the transmissibility of viruses in appropriate animal models. In this section, an overview of GoF approaches in this phenotypic category is provided and the scientific outcomes and/or products of each approach are described.

15.3.1.1 Serial Passaging of Viruses in Mammalian Cells or Animals

Serial passaging of viruses in mammalian cells in laboratory animals selects for viruses with enhanced growth in cells or enhanced infectivity to animals, respectively. This type of serial passaging experiment involves "forced" passaging, meaning that the experimenter directly transfers infected material, in the form of cell culture supernatant or homogenates of infected tissue, to the subsequent cell culture dish or animal. Forced serial passaging is carried out for two purposes: (1) to identify mutations that arise during adaptation of animal influenza viruses (i.e., avian and swine viruses) to mammals, which provides a foundation for follow-up studies investigating the evolutionary mechanisms driving adaptation to mammalian hosts and the mechanistic basis of mammalian adaptation, and (2) to develop an mouse model for the study of a particular virus.

15.3.1.2 Serial Passaging of Viruses in Mammalian Cells or Animals with Selection for Transmission

Serial passaging of viruses in animals with selection for transmission leads to the generation of viruses with enhanced transmissibility in mammals. This type of serial passaging experiment can involve selection for contact transmission, during which the primary (directly inoculated) and secondary hosts are co-housed, or for airborne transmission, during which the primary and secondary hosts are separately housed in special isolator cages that prevent direct contact between animals but allow for air exchange between cages. These studies seek to identify mutations that are sufficient to enhance transmissibility, which provides a foundation for follow-up studies that investigate the mechanistic basis of transmissibility in mammals.

15.3.1.3 Forward Genetic Screen to Identify Genetic Traits That Enhance the Fitness/Transmissibility of Viruses in Mammals

Forward genetic screens involve random mutagenesis of genetic regions predicted to contribute to fitness/transmissibility or comprehensive reassortment of parental gene segments from two viruses, followed by characterization of the fitness or transmissibility of mutants in appropriate mammalian model systems to select for mutant viruses with enhanced fitness/transmissibility. Sequencing emergent viruses enables the identification of mutations or gene segments that enhance the fitness/transmissibility of

viruses, which provides a foundation for follow-up studies that investigate the mechanistic basis of transmissibility in mammals.

15.3.1.4 Targeted Genetic Modification of Viruses to Introduce Traits That Are Expected to Enhance Fitness/Transmissibility in Mammals

Targeted genetic modification of viruses, namely site-directed mutagenesis and/or reassortment, to introduce genetic traits that are expected to enhance the fitness/transmissibility of viruses followed by characterization of the fitness or transmissibility of mutants in appropriate mammalian model systems may lead to the generation of viruses with enhanced fitness/transmissibility in mammals. This approach is performed for two purposes: (1) to determine whether a previously characterized underlying genetic or phenotypic trait, such as a preference for binding to α2,6 sialic acid receptors, contributes to the complex phenotypes of mammalian adaptation or transmissibility and (2) to confirm that a particular mutation or gene segment is necessary and sufficient to enhance the fitness/transmissibility of viruses in appropriate model systems. Notably, genetic traits that are associated with mammalian adaptation/transmissibility may be discovered through GoF approaches or alt-GoF approaches. As above, this information provides a foundation for follow-up studies investigating the mechanistic basis of mammalian adaptation and transmissibility.

15.3.2 Overview of the Potential Benefits of GoF Approaches That Enhance the Fitness/Transmissibility of Influenza Viruses

15.3.2.1 Scientific Knowledge

GoF approaches have potential to benefit several aspects of scientific knowledge about the ability of animal influenza viruses to adapt to efficiently infect and transmit between humans. GoF approaches can provide insight into: (1) whether animal influenza viruses can acquire the capacity for airborne transmissibility between mammals, (2) the evolutionary mechanisms driving adaptation of animal influenza viruses to efficiently infect and transmit between mammals, and (3) the mechanistic basis of mammalian adaptation and transmissibility of animal influenza viruses.

15.3.2.2 Surveillance

GoF approaches that lead to the identification of molecular markers for mammalian adaptation and transmissibility between mammals have the potential to inform the interpretation of wildlife, agricultural animal, and public health surveillance information. Specifically, determining the presence (or absence) of particular mutations or of amino acid substitutions at particular sites is one aspect of evaluating the risk posed by circulating animal influenza viruses. Risk assessments based on evaluation of genetic surveillance data, as well as other types of data, then inform decision-making related to public health preparedness for novel influenza outbreaks, as discussed below.

15.3.2.3 Vaccines

GoF approaches have the potential to benefit the development of pre-pandemic vaccines. Specifically, pandemic risk assessments, which can be informed by GoF research (see Section 16.3.2.2), may trigger the development of candidate vaccine viruses based on high-risk viruses, as well as subsequent stages of the pre-pandemic vaccine production pipeline (e.g., manufacturing of clinical lot material, conducting human clinical trials, and stockpiling vaccine).

15.3.2.4 Therapeutics

A lack of knowledge about whether existing therapeutics will be effective against future pandemic strains hampers preparedness planning. GoF-generated viruses that are transmissible between ferrets may mimic pandemic variants of that HA subtype better than wild type viruses. Thus, testing whether existing therapeutics are capable of mitigating disease caused by GoF strains could inform pandemic preparedness planning. Researchers have also suggested that these experiments could stimulate the development of new therapeutics, in the event that existing therapeutics are found to be ineffective against GoF strains. However, the relevance and utility of this information is severely constrained by several sources of uncertainty, including a lack of knowledge about whether ferret-transmissible viruses are more transmissible in humans, whether laboratory-generated transmissible viruses behave similarly to those that could arise in nature, and other factors. Given this uncertainty, dedication of resources to developing therapeutics targeting hypothetical future pandemic viruses is unlikely. Thus, this putative benefit to the development of therapeutics is not considered in this report.

15.3.2.5 Diagnostics

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[1427]

15.3.2.6 Informing Policy Decisions

GoF approaches that lead to the identification of molecular markers for mammalian adaptation and transmissibility between mammals contribute to assessments of the pandemic risk posed by circulating animal influenza viruses, which are based on genetic surveillance data and several other types of data (e.g., epidemiologic data, phenotypic data, etc.). These assessments inform policy decisions related to public health preparedness for novel influenza outbreaks, including whether to develop and publicize messaging about risk factors for contracting animal influenza infections and practices for mitigating risks, whether to enhance surveillance of animals, and whether to develop pre-pandemic vaccines.

15.3.2.7 Economic Benefits

Pandemic risk assessments inform prioritization of resources for pandemic preparedness. Specifically, evaluating the relative risk posed by different influenza viruses helps decision-makers allocate limited funds to pandemic preparedness efforts, such as the development of pre-pandemic vaccines targeting high-risk viruses. This prioritization may improve the efficiency of government spending on influenza pandemic preparedness. Economic benefits were not explicitly evaluated in this report.

15.3.3 Benefits of GoF to Scientific Knowledge

In this section, the ability of GoF approaches to address three key outstanding questions related to influenza virus adaptation and transmission in humans is evaluated:

- *Can* animal influenza viruses become transmissible between humans?

[1427] New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

- *How* do animal influenza viruses adapt to and become transmissible between humans? What selective pressures drive adaptation and the evolution of efficient transmissibility, and what is the order of acquisition of new genetic/phenotypic traits that are needed for adaptation/transmissibility?

- *What* is the mechanistic basis of adaptation and transmission in humans? What viral factors are involved, and what phenotypic changes must occur in order for an animal influenza virus to adapt to efficiently infect, cause disease, and transmit in mammals?

Viral fitness and transmissibility in any model system are complex phenotypes that arise through the cumulative effects of multiple underlying phenotypes, such as specificity for a particular type of cell surface receptor and the ability to replicate within a particular temperature range. Generally, the biological process of acquiring efficient transmissibility in a new host species can be viewed as the result of two interdependent steps. First, the virus must be able to infect a new host, which depends on underlying traits that contribute to mammalian adaptation, and second, the virus must be able to get out of the primary host and infect a secondary host. Because the property of transmissibility depends on phenotypes underlying both adaptation and transmission and because similar experimental approaches are used to study both complex phenotypes, GoF experiments that enhance adaptation and transmissibility are discussed together in this section.

The evolutionary mechanisms driving adaptation of viruses to new hosts and the acquisition of efficient transmissibility, as well as the underlying genetic and phenotypic traits that enable efficient infection and transmission in human populations, are poorly understood. Several phenotypes have been shown to be associated with mammalian adaptation and transmissibility, including a preference for HA binding to cell surface receptors decorated with α2,6 sialic acid moieties (versus "avian-like" α2,3 sialylated receptors), the ability of the viral polymerase complex to function at lower temperatures, and an increase in HA stability. However, considerable gaps in knowledge remain about the molecular basis of each phenotype and the role of each phenotype in adaptation/transmissibility, and as-yet-undiscovered viral factors and phenotypic changes are likely to contribute to the acquisition of efficient transmissibility in mammals. Furthermore, the potential for animal influenza strains to evolve efficient transmissibility in humans is not understood.

15.3.3.1 Scientific Knowledge Gap 1: Can Animal Influenza Viruses Become Transmissible Between Humans?

15.3.3.1.1 Benefits and Limitations of GoF Approaches

Several GoF approaches can lead to the generation of transmissible viruses, including deliberate genetic modification of viruses and serial passaging of viruses in animals with selection for transmission. Collectively, these approaches definitively demonstrate that a virus can acquire the capacity to transmit between laboratory animals in an experimental setting. Notably, this approach can be applied to strains that have not yet caused infections in human populations as well as strains that have caused human infections but do not yet efficiently transmit in humans. The key limitations of this approach are that observations in animal models may not translate to humans and that the adaptive changes observed in the laboratory may not be possible in nature.

15.3.3.1.2 Benefits and Limitations of Alt-GoF Approaches

Characterizing the transmissibility of wild type isolates in representative animal models represents an alternative approach for addressing whether animal influenza viruses display the capacity for transmission

between mammals. However, this approach is inherently reactive– that is, it can effectively answer whether a virus is transmissible but cannot shed light on whether a virus has the potential to become transmissible. As above, observations in animal models may not translate to humans.

15.3.3.1.3 Summary – Benefits of GoF approaches Relative to Alt-GoF Approaches

GoF approaches are uniquely capable of *proactively* assessing the potential for *any* animal influenza viruses to acquire enhanced fitness and transmissibility in mammals. Notably, the relevance of this information for human populations depends on the suitability of animal models as well as whether laboratory-acquired mutations can arise in nature, both of which are unknown (Table 15.14).

Table 15.14. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits—*Can* a Virus Acquire Efficient Transmissibility in Appropriate Animal Models?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1 [1][a]: Targeted genetic modification to introduce genetic changes expected to contribute to transmissibility **GoF #2 [2]**: Forward genetic screen to introduce genetic changes that may contribute to transmissibility **GoF #3 [3]**: Serial passaging with selection for transmission	• Determine whether virus **can** acquire the capacity for transmission in appropriate animal models ○ Proactive – can be performed using viruses that do not yet transmit between humans in nature	• Narrow breadth – Results may not generalize to other virus strains • Artificiality – Adaptive changes observed in the laboratory may not be likely or possible in nature
Alt-GoF #1 [3]: Characterization of wild type viruses[b]	• Determine whether virus **is** transmitted in appropriate animal models	• Narrow breadth – Results may not generalize to other virus strains • Reactive – Limited to analysis of viral isolates that already exist in nature ○ Results from single round of selection may not reflect virus capacity for evolution of transmissibility for strains that have not yet caused infections in human populations or strains that have caused human infections but do not readily transmit in humans

[a] *GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets reference the order in the landscape tables (Supplementary Information).*

[b] *Note that to date, animal-origin viruses that efficiently transmit between humans have not been discovered in nature. If available, characterization and phenotypic analysis of wild type isolates represents a viable alternative approach.*

15.3.3.2 Scientific Knowledge Gap 2: How Do Animal Influenza Viruses Adapt to and Become Transmissible in Humans?

15.3.3.2.1 Benefits and Limitations of GoF Approaches

Serial passaging of animal influenza viruses in appropriate animal models to select for mammalian adaptation and transmission, a GoF approach, provides insight into the mechanisms underlying adaptation to mammals and the evolution of transmissibility. This approach is flexible, in that the method of passaging (i.e., by direct inoculation, direct contact transmission, or airborne contact transmission) and the tissue source used for forced passaging can be adjusted to study different modes of transmission. Sequencing of isolates at multiple stages of passaging enables determination of the order and rate of acquisition of adaptive traits, and follow-up studies elucidate how those genetic and phenotypic changes influence other viral phenotypes. Comparing the sequences and phenotypes of viral isolates from different tissues, different time points during the course of infection, and between the primary (directly inoculated) and the secondary hosts can provide additional insight into the tissue-dependence of adaptation, the rate of intra- and inter-host adaptation, and the selection pressures and viral population dynamics during transmission, respectively. Notably, the adaptive changes that occur in the lab environment under forced selection may not be relevant or possible during natural evolution, may not mimic adaptation and transmission in humans, and may selectively represent the evolutionary course possible for the limited number of viruses studied.

Serial passaging, as well as the alt-GoF methods described below, provides information about the genetic traits that are associated with the acquisition of enhanced fitness and transmissibility in mammals. However, to confirm which of these changes are *necessary* and *sufficient* to enhance fitness and transmissibility, targeted mutagenesis must be used to re-introduce mutations into parental strains followed by characterization of the infectivity/transmissibility of mutant strains. Targeted mutagenesis also enables determination of how the order of acquisition of genetic changes influences other viral phenotypes, such as replicative fitness, which has implications for the likelihood that these traits can arise in nature.

15.3.3.2.2 Potential Benefits and Limitations of Alt-GoF Approaches

Several alt-GoF approaches can also address how influenza viruses evolve to efficiently infect and transmit in humans. First, the comparison of sequences from closely related human and animal isolates enables the identification of the origin and evolutionary rate of genetic changes among circulating viruses, which can provide information on selection pressures and diversity among viruses in different hosts. The fact that this approach examines the natural course of adaptation and underlying mechanisms of infection and transmission of viruses *in humans* is a strength relative to GoF approaches and other alternatives that depend on the suitability of animal models in an artificial environment as representative of human disease. An additional strength of the comparative sequence analysis method is the ability to analyze genetic features across broad data sets including many viral isolates.

This approach suffers from several significant limitations. The use of comparative sequence analysis is feasible only if human-adapted and transmissible viruses have arisen in nature, but to date, animal influenza viruses have limited capacity to infect and transmit in humans. Analysis of the few animal-origin spillover infections may however inform evolution of adaptive traits. The success of this approach depends on the quality and availability of surveillance data. In particular, the noisiness of comparative sequence analysis due to high genetic diversity among influenza viruses practically limits this approach to the examination of genetic regions known to be important for adaptation and transmissibility. The

identification of precursor strains that are closely related to zoonotic or human-adapted viruses strengthens the utility of this approach by reducing the genetic diversity between compared strains, however precursor-spillover paired strains have not been identified in all cases. Moreover, available sequences may not capture all of the critical adaptive steps and cannot identify traits that were lost or negatively selected during adaptation (i.e., evolutionary pathways not taken). Thus this approach may provide less depth of information about how positively and negatively selected genetic traits interact to determine fitness in distinct host populations and during transmission.

Analysis of viruses that have emerged from avian or mammalian reservoirs to become transmissible in other mammalian species represents another surveillance-based approach for studying the mechanisms underlying adaption to mammals during interspecies transmission. The recent emergence of animal transmissible influenza viruses in other mammals (e.g., an avian-origin H3N2 canine influenza virus that emerged in dogs in the mid-2000s) enables the study of the full evolutionary pathway for cross-species acquisition of efficient transmissibility. This approach is subject to the same limitations as comparative sequence analysis of human and animal isolates, with the additional caveat that adaptation to other mammals may occur through different pathways and mechanisms than in humans.

Phenotypic characterization of wild type viruses by evaluating infectivity and transmissibility in appropriate model systems is another alt-GoF approach for studying the evolution and mechanisms of adaptation/transmissibility. This approach allows for the generation of detailed information about intra- and inter-host evolutionary dynamics and can uncover both negatively and positively selected mutations. However, it is limited to observation of adaptive changes over a single round of transmission, effectively limiting the time and selective pressure under which adaptation occurs. It should be noted that in some cases, mutations associated with adaptation and transmissibility can be generated *in vivo* within a single round of transmission. Any animal influenza viruses that are highly attenuated in representative animal models or are incapable of establishing infection are not suitable for this approach. Furthermore, this approach is limited by its narrow breadth and depends on the suitability of the animal models used for characterization.

15.3.3.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The scientific knowledge benefits and limitations of all GoF and alt-GoF approaches discussed in this section, with respect to the ability of each approach to provide insight into the evolution of fitness and transmissibility in mammals, are summarized in Table 15.15. Taken together, GoF approaches are uniquely capable of providing in-depth information about the evolution of mammalian fitness/transmissibility in *any* animal influenza virus strain. In addition, GoF approaches are uniquely capable of demonstrating the order(s) of acquisition of genetic changes that are necessary and sufficient to lead to enhanced fitness/transmissibility in mammals. However, the relevance of information derived from GoF approaches is contingent upon how well animal models represent human disease and how well the lab environment mimics natural evolution.

For those wild type strains that are naturally capable of productively infecting laboratory animals used for transmission studies, simply characterizing the transmissibility of a strain in animals, an alt-GoF approach, has the potential to generate similarly in-depth information. However, a single round of transmission may be insufficient for relevant adaptive changes to accrue or may reveal only part of the adaptive process, which further lessens the relative utility of this alt-GoF approach. Surveillance-based approaches, including comparison of human and animal isolates and comparison of animal isolates from different species, are uniquely capable of reporting on the real-world evolution of a variety of strains, thus complementing two shortcomings of GoF approaches. Though results gleaned from comparative analysis of human and animal isolates are directly translatable to humans, the fact that animal influenza virus strains that efficiently transmit in humans have not been observed in nature precludes use of this approach

for the study of transmissibility in particular. While case studies of interspecies transmission events exist, the translatability of that information to the evolution of human adaptive traits is uncertain.

Table 15.15. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits—*How* Do Animal-Origin Viruses Adapt to and Become Transmissible in Humans?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1 [4][a]: Targeted genetic modification to introduce genetic changes expected to contribute to adaptation/transmissibility	• Provide insight into evolutionary mechanisms driving adaptation/transmissibility • Determine order of acquisition of genetic changes that are **necessary** and **sufficient** to enhance adaptation/transmissibility ○ Determine how rate and order of acquisition of genetic changes affects other viral phenotypes • Proactive - can be performed using viruses that do not yet transmit between humans in nature	• Translatability – Results may not translate to adaptation in humans

GoF #2 [3]: Serial passaging with or without selection for transmission	• Provides **in-depth** insight into evolutionary mechanisms driving adaptation/transmissibility
	  ○ Captures all adaptive steps
	  ○ Identifies positively and negatively selected traits
	  ○ Evaluates adaptation over a long time period and under high selective pressures
	• Determine how rate and order of acquisition of genetic changes affects other viral phenotypes
	• Proactive - can be performed using viruses that do not yet transmit between humans in nature
	• Uncovers **previously unidentified** genetic and phenotypic traits mediating evolution
		• Narrow breadth – Results may not generalize to other virus strains
		• Artificiality – Adaptive changes observed in the laboratory may not be representative of evolution in nature
		• Translatability – Results may not translate to adaptation in humans

Table 15.15. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits—*How* Do Animal-Origin Viruses Adapt to and Become Transmissible in Humans?		
Experimental Approach	**Benefits**	**Limitations**
Alt-GoF #1 [1]: Comparative sequence analysis of human and animal isolates	• Provide insight into evolutionary mechanisms driving adaptation/transmissibility o Identify the origin and evolutionary rate of genetic changes among circulating viruses, o Provides information on the **natural** evolutionary process, o **Directly** translates to **human** adaptation and disease, and o Analyzes **broad** data sets applicable to many strains	• Lack of correlate[b] – Animal-origin viruses have limited capacity to infect and transmit in humans, limiting the availability of suitable data • Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Limited by the quality and availability of existing surveillance data o Consensus sequences may not capture extent of viral diversity • Static – Cannot identify lost or negatively selected traits, and intermediate adaptive events may not be captured • Associative – Information produced is correlative, not causative

Table 15.15. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals

Scientific Knowledge Benefits—*How* Do Animal-Origin Viruses Adapt to and Become Transmissible in Humans?

Experimental Approach	Benefits	Limitations

Alt-GoF #2 [2]: Comparative sequence analysis of animal isolates from two species	• Provide insight into evolutionary mechanisms driving adaptation/transmissibility
	  ○ Identify the origin and evolutionary rate of genetic changes among circulating viruses.
	  ○ Provides information on the **natural** evolutionary process
	  ○ Analyzes **broad** data sets applicable to many strains
		• Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important
		• Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty
		• Limited by the quality and availability of existing surveillance data
		  ○ Consensus sequences may not capture extent of viral diversity
		• Static – Cannot identify lost or negatively selected traits, and intermediate adaptive events may not be captured
		• Associative – Information produced is correlative, not causative
		• Translatability – Results may not translate to adaptation in humans
		  ○ Whether animals under study are representative models for human disease has not been established

Alt-GoF #3 [3]: Characterization of wild type viruses	• Provides insight into evolutionary mechanisms driving adaptation/transmissibility
	  ○ Captures all adaptive steps
	  ○ Identifies positively and negatively selected traits
	• Determine how rate and order of acquisition affects other viral phenotypes
	• Proactive - can be performed using viruses that do not yet transmit between humans efficiently in nature
	• Uncovers **previously unidentified** genetic and phenotypic traits mediating evolution
		• Narrow breadth – Results may not generalize to other virus strains
		• Translatability – Results may not translate to adaptation in humans
		• Associative – Information produced is correlative, not causative
		• Limited to use of viruses that can productively infect representative animal models
		• Single (vs. multiple) round of infection/transmission limits the time for evolution and the amount of applied selection pressure
		  ○ May be insufficient time for relevant evolutionary changes to accrue
		  ○ May capture only part of the adaptive process

Table 15.15. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits—*How* Do Animal-Origin Viruses Adapt to and Become Transmissible in Humans?		
Experimental Approach	**Benefits**	**Limitations**
[a] *GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets reference the order in the landscape tables (Supplementary Information).*		
[b] *Note that to date, animal-origin viruses that efficiently transmit between humans have not been discovered in nature. If available, comparative sequence and phenotypic analysis represents a viable approach for identification of genetic markers associated with human adaptation/transmissibility.*		

15.3.3.3 Scientific Knowledge Gap 3: What Are the Genetic and Phenotypic Traits That Result in Adaption and Transmission in Humans?

15.3.3.3.1 Potential Benefits and Limitations of GoF Approaches

Several GoF approaches can be used to discover the genetic and phenotypic markers underlying mammalian adaptation and transmission of animal influenza viruses, including:

- Targeted genetic modification to introduce novel genetic changes that are expected to contribute to adaptation and transmission in mammals by either site-directed mutagenesis or targeted reassortment (often between animal and human seasonal strains),
- Forward genetic screens involving random mutagenesis or comprehensive reassortment followed by selection for mammalian infectivity, transmissibility, or underlying phenotypes, and
- Serial passaging in appropriate animal models or mammalian cells to select for mammalian adaptive or transmissible traits.

Collectively, these approaches enable the identification of genetic changes that are sufficient to confer enhanced fitness in cell culture model systems or infectivity and transmissibility in animal models, which provides a foundation for follow-up biochemical, cell biological, and structural studies that elucidate associated phenotypic changes. Serial passaging has the potential to uncover *novel* genetic and phenotypic markers that contribute to adaptation/ transmissibility. In contrast, because forward genetic screens involving random mutagenesis typically focus on regions that are suspected or known to play a role in phenotypes underlying adaptation/transmissibility, this approach can discover novel *genetic* markers for adaptation/transmissibility only. The targeted genetic modification approach is limited to the investigation of genetic traits and underlying phenotypes that are suspected to contribute to adaptation/transmissibility (e.g., determining whether altering sialic acid receptor binding specificity contributes to transmissibility). Targeted genetic modification is also used to confirm that particular mutations or gene segments are *necessary* and *sufficient* to enhance infectivity or transmissibility in mammals. The use of *in vitro* model systems is limited to the investigation of phenotypes underlying adaptation and transmissibility, such as replicative fitness and sialic acid receptor specificity. Moreover, the results derived from these studies may not be recapitulated in the complex environmental pressures encountered in a host. The relevance of both *in vitro* and *in vivo* approaches depends on whether mechanisms underlying adaptation to cell culture and animal models are representative of those in humans, and results gleaned from the study of one or a few strains may not be recapitulated in different genetic contexts.

15.3.3.3.2 Benefits and Limitations of Alt-GoF Approaches

Several alt-GoF approaches can be used to uncover genetic and phenotypic traits underlying adaptation and transmission in mammals. First, comparing the sequences of human and animal isolates enables the identification of genetic changes that are associated with human adaptation and transmissibility. Unlike the GoF approaches described above, this approach has the potential to directly identify human-adaptive traits and may be more likely to uncover conserved traits through analysis of a large number of strains. However, the fact that no animal influenza viruses that efficiently transmit in humans have been observed in nature precludes the use of this approach to identify mechanisms underlying transmissibility. For the discovery of mammalian adaptive traits, the success of this approach depends on the quality and availability of surveillance data. In particular, the fact that nearly all published sequences represent consensus sequences means that the presence of rare adaptive traits that arise in human cases may not be captured in the data. Finally, the extensive genetic diversity within circulating virus populations and

among viruses isolated from humans makes discerning distinct genetic traits that are likely to contribute to fitness and transmissibility in humans relative to animals difficult. Namely, the "noise" associated with sequences comparisons obscures the discovery of relevant features that distinguish human versus animal isolates, which practically limits this approach to the investigation of traits or regions previously known to be important for adaptation.

Comparing the sequences of evolutionarily related isolates from different animal species represents another surveillance-based approach for identifying genetic traits that are associated with mammalian adaptation and transmissibility. Importantly, because avian-origin flu viruses that are airborne or contact transmissible exist in circulation in several mammals including seals, horses, and dogs, this approach is currently feasible for the study of transmissibility. In addition to the limitations above, mechanistic insight gleaned through this approach may not translate to the adaptation of animal influenza viruses to humans.

Phenotypic characterization of wild type viruses in appropriate animal models is another alt-GoF approach that complements the use of surveillance data to study mechanisms underlying mammalian adaptation and transmissibility. Specifically, comparing the sequences of wild type viruses with varied levels of fitness and transmissibility enables the identification of genetic traits associated with fitness/transmissibility. This approach is limited to the study of viruses that can productively infect and transmit between animal models for adaptation/transmission. Notably, very few natural animal-origin viruses are capable of transmission in ferrets and many are not able to efficiently cause disease in representative animal models. Similarly to GoF approaches, genetic and phenotypic traits uncovered through this approach may not translate to human-adapted viruses and may only be applicable to the limited number of strains analyzed.

Loss of Function (LoF) approaches, genetic screens that utilize random mutagenesis or targeted genetic modification to identify genetic changes that attenuate fitness and transmission in mammals, can provide information about genetic and phenotypic traits that contribute to transmissibility. Targeted LoF can also be used to confirm necessary genetic or phenotypic traits by determining that mutations attenuate fitness or transmission, but cannot identify traits that lead to enhanced transmission. This approach suffers from several significant limitations. First, LoF studies can be performed only using transmissible seasonal or pandemic viruses, and insights may not translate to animal influenza viruses. Second, because of the high mutation rate of influenza viruses, LoF mutations that attenuate transmissibility may revert during the single round of passage that is needed to characterize the transmissibility of the mutants (which represents a selection step). Third, because many mutations attenuate transmission for trivial reasons, for example mutations that compromise viability, discovering traits that directly contribute to transmissibility may be difficult using a LoF approach. Finally, although in principle LoF screens can be performed after random mutagenesis to discover new genetic elements important for transmission, the resource intensive nature of transmission studies in ferrets practically limits these studies to the investigation of a few, known targets.

Several *in vitro* virus-free methods can be used to investigate phenotypes underlying adaptation and transmissibility. Comparative sequence analysis of viral proteins with different phenotypic properties can then enable the identification of mutations that are associated with relevant phenotypic changes, while forward genetic screens can be used to identify novel *genetic* traits that contribute to underlying phenotypes. Additional characterization involves the use of biochemical assays (e.g., characterizing the acid stability of the HA protein) and crystallographic resolution of the structures of virus-host protein complexes can provide insight into the functional and biophysical basis of underlying phenotypes. The use of targeted modification of viral gene segments in isolation can also effectively confirm the *necessary* and *sufficient* genetic traits that alter an underlying phenotype. Though the simplicity and relatively high-throughput nature of these methods renders them appealing as a screening approach for the discovery and confirmation of *novel* genetic traits that contribute to adaptation/transmissibility, these approaches are

inherently limited to the characterization of phenotypes (and genetic traits in the case of targeted modification) previously identified in other experiments. An additional drawback is that results gleaned from studying the behavior of a viral protein or phenotype in isolation may not be recapitulated in the context of the full virus or *in vivo*. Moreover, although fairly rapid phenotypic assays have been developed for the study of phenotypic traits known to be associated with adaptation/transmissibility, assays to study phenotypic traits may be unreliable or unavailable for future phenotypes of interest.

Structure-based modeling approaches, an *in silico* method, may also be used to predict the effects of mutations on phenotypes underlying adaptation/transmissibility. This approach is critically limited by the capabilities and accuracy of existing models, and as such any conclusions may not be consistent in the context of the full virus.

Finally, several alt-GoF approaches focus on identifying host factors and host-virus interactions that are associated with mammalian adaptation, which may provide indirect insight into viral mechanisms underlying cross-species adaptation. Specifically, *in vitro* proteomic (e.g., mass spectrometry) and genomic screens (e.g., RNAi screen) utilizing both virus-free and cell culture-based infection systems are used to identify host factors that interact with virus proteins of interest or that are critical for underlying phenotypes such as viral replication. These approaches complement the identification of viral proteins/phenotypes underlying adaptation to new hosts. However, the breadth of proteomic approaches is limited in that screens typically focus on a single viral protein, and both genomic and proteomic screens can identify host proteins that may not be functionally relevant or may play minor roles in the viral life cycle.

Another type of alternative approach involves the use of attenuated viruses for GoF methods, as a risk mitigation strategy. Four types of attenuated viruses have been used for such studies: (1) reassortants with surface protein gene segments from seasonal influenza viruses, to which the general population has pre-existing immunity, (2) reassortants with lab-adapted viruses (e.g., PR8), (3) strains which have virulence factors altered or deleted (e.g., deletion of the multi-basic cleavage site in HPAI HA sequences), and (4) strains which have incorporated binding sites for microRNAs (miRNAs) that are expressed in humans but not an animal model of interest, and therefore are replication-competent in experimental animals but not humans (termed "molecular biocontainment").[1428] Results gleaned through use of the first three types of attenuated viruses may be of limited informational value because complex, multi-genic traits depend on genetic context (a phenomenon called epistasis), and results may not be recapitulated in the context of the wild type virus. Differences in disease pathogenesis, which critically influences the biological processes of adaptation and transmission, further compromise the relevance of results gained through the use of attenuated strains. In addition, several factors limit the range of information that can be generated using attenuated strains. First, seasonal reassortant strains can be used to study the role of genes that encode internal factors (e.g., polymerase and nucleoprotein, etc.) only, while lab-adapted reassortants are limited to the study of proteins donated by the wild type strain. Second, lab-adapted reassortants cannot cause disease or transmit in ferrets and thus cannot be used to study airborne transmissibility in this model system. Other types of attenuated strains, such as strains in which the multi-basic cleavage site has been deleted, may not be suitable for *in vivo* studies. Finally, although the microRNA-based molecular biocontainment strategy is considered promising by the influenza research community, only two such engineered strains have been created to date, which incorporate miRNA target sites that permit replication in ferrets but restrict replication in humans and mice (i.e., miR-192). Neither of these engineered strains has been extensively characterized with respect to infection and transmission dynamics in ferrets or permissive cell lines. Additional research is needed to determine whether and to what extent the engineered strains serve as functional proxies for their cognate WT strains, before these strains can be

[1428] Langlois RA *et al* (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844-847

widely used to probe scientific questions about mammalian adaptation and transmission of influenza viruses. In addition, because the purpose of this miRNA strategy is to restrict virus replication in people, this strategy is not suitable for studies using human cell lines, limiting its utility for *in vitro* studies investigating phenotypes underlying mammalian adaptation and transmissibility.

15.3.3.3.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Tables 15.16 and 15.17 summarize the benefits and limitations of GoF and alt-GoF approaches that provide insight into the mechanisms underlying the fitness and transmissibility of influenza viruses in mammals. Taken together, GoF approaches are uniquely capable of identifying novel genetic and phenotypic traits underlying mammalian adaptation and transmissibility in *any* animal influenza virus strain of interest. Furthermore, targeted genetic modification of viruses to introduce genetic traits associated with mammalian adaptation/transmissibility is uniquely capable of demonstrating that particular genetic markers are *necessary* and *sufficient* for mammalian adaptation and transmissibility across multiple virus contexts. Given the importance of genetic context for influenza biology, this approach critically strengthens the certainty of scientific knowledge about mechanisms underlying mammalian adaptation and transmissibility. However, results gleaned from cell culture and animal model studies may not translate to human disease. Notably, most attenuated strains cannot be used to study mechanisms underlying airborne transmission because these strains do not efficiently infect ferrets. Additionally, attenuation alters disease pathogenesis and compromises the utility of the information gleaned through studies using other model systems. Although microRNA-based strategies for "molecular biocontainment" have shown promise for transmission studies in ferrets, further research is needed to determine whether these strains will serve as reliable proxies for a wide variety of wild type viruses. In addition, miRNA-based strategies cannot be used for studies involving human cell lines, limiting their utility for *in vitro* studies examining phenotypes underlying mammalian adaptation and transmissibility.

Characterizing wild type viruses, an alt-GoF approach, also has the potential to uncover previously unknown traits. However, the fact that this approach cannot be used to study animal influenza viruses that do not productively infect laboratory animals and that relevant changes may not arise during a single round of transmission renders it less useful than GoF approaches. LoF approaches have limited utility for broad and unbiased identification of necessary genetic and phenotypic traits due to their inefficiency and the fact that mechanisms underlying transmissibility of seasonal/pandemic viruses may not translate to animal influenza viruses. The simplicity and relative high-throughput nature of *in vitro*, virus-free systems renders them appealing for the discovery of novel genetic traits that alter *known* phenotypes underlying mammalian adaptation/transmissibility, but properties observed may not be recapitulated during the complete viral life cycle.

Unlike GoF methods, the use of human and animal surveillance data for the discovery of genetic markers associated with adaptation/transmission directly translates to human disease and has strength in numbers as it analyzes genetic traits across large data sets. Critically, this approach cannot be used for studying transmissibility because animal or zoonotic viruses that efficiently transmit in humans have not been observed in nature. Analysis of sequences spanning avian to mammalian adaptation events enables the identification of "real-world" markers associated with mammalian adaptation/transmissibility but may not translate to human-adapted viruses. For both surveillance-based approaches, shortcomings in the quality and availability of surveillance data compromise the feasibility of this approach and the relevance of any findings.

Finally, host-focused approaches, such as proteomic and genomic screens, cannot supplant the identification of viral adaptation/transmissibility traits but rather complement GoF approaches by identifying host factors that contribute to those processes.

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1a [1,4,5][a]: Targeted genetic modification to introduce genetic changes expected to contribute to transmissibility (*in vivo*)	• Identifies genetic and phenotypic traits that are **necessary** and **sufficient** for adaptation to mammals or enhanced transmissibility (i.e., provides causative data) • Gain insight into phenotypes underlying adaptation/transmissibility • Proactive - can be performed using viruses that do not yet transmit between humans in nature • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from representative animal models may not translate to mechanisms underlying transmissibility in humans • Bias – Limited to investigation of previously identified phenotypic *or* genetic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved
GoF #1b [1,4,5]: Targeted genetic modification to introduce genetic changes expected to contribute to transmissibility (*in vitro*)	• Identifies genetic and phenotypic traits that are **necessary** and **sufficient** for viral fitness (i.e., provides causative data) • Gain insight into phenotypes underlying fitness • Proactive - can be performed using viruses that do not yet transmit between humans efficiently in nature • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Limited to the investigation of viral fitness, which is one component of mammalian adaptation and transmissibility • Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from cell culture models may not translate to humans • Bias – Limited to investigation of previously identified phenotypic *or* genetic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?		
Experimental Approach	**Benefits**	**Limitations**
GoF #2a [2]: Forward genetic screen to introduce genetic changes that may contribute to transmissibility, followed by testing *in vivo*	• Identifies **novel** genetic traits that are sufficient for mammalian adaptation/enhanced transmissibility • Gain insight into phenotypes underlying adaptation/transmissibility • Proactive - can be performed using viruses that do not yet transmit between humans efficiently in nature	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from representative animal models may not translate to mechanisms underlying transmissibility in humans • Bias – Limited to investigation of previously identified *phenotypic* traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Associative – Information produced is correlative, not causative
GoF #2b [2]: Forward genetic screen to introduce genetic changes that may contribute to phenotypes underlying transmissibility, followed by testing *in vitro*	• Identifies novel genetic traits that are sufficient to enhance viral fitness • Gain insight into phenotypes underlying fitness • *In vitro* methods can be used to screen a larger number of mutants than *in vivo* methods • Proactive - can be performed using viruses that do not yet transmit between humans efficiently in nature	• Narrow breadth – Results may not generalize to other virus strains • Limited to the investigation of viral fitness, which is one component of mammalian adaptation and transmissibility • Translatability – Results from cell culture models may not translate to humans • Bias – Limited to investigation of previously identified *phenotypic* traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Associative – Information produced is correlative, not causative
GoF #3a [3]: Serial passaging with selection for transmission, use of animal models (*in vivo*)	• Identifies novel genetic and phenotypic traits that are sufficient for mammalian adaptation/enhanced transmissibility • Gain insight into phenotypes underlying adaptation/transmissibility • Proactive - can be performed using viruses that do not yet transmit between humans efficiently in nature	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from representative animal models may not translate to mechanisms underlying transmissibility in humans • Associative – Information produced is correlative, not causative

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?		
Experimental Approach	**Benefits**	**Limitations**
GoF #3b [3]: Serial passaging with selection for transmission, use of cell culture models (*in vitro*)	• Identifies novel genetic and phenotypic traits that are sufficient to enhance viral fitness • Gain insight into phenotypes underlying fitness • Proactive - can be performed using viruses that do not yet transmit between humans efficiently in nature	• Narrow breadth – Results may not generalize to other virus strains • Limited to the investigation of viral fitness, which is one component of mammalian adaptation and transmissibility • Translatability – Results from cell culture models may not translate to humans • Associative – Information produced is correlative, not causative
Alt-GoF #1 [1]: Comparative sequence analysis of human and animal isolates	• Identifies genetic traits that are associated with human adaptation/transmissibility o Comparison of genetically similar viruses, such as precursor strains/spillover pairs, can result in the identification of previously unknown genetic traits that are associated with human adaptation o Depending on the size of analysis and strength of association some traits can be considered "causally" linked o **Directly** translates to **human** adaptation and disease o Analyzes **broad** data sets applicable to many strains • Gain insight into the prevalence and distribution of genetic traits o Can infer functional generalizability of genetic traits (i.e., whether they do or do not behave similarly in different genetic contexts)	• Lack of correlate[b] – Animal-origin viruses have limited capacity to infect and transmit in humans, limiting available data • Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Limited by the quality and availability of existing surveillance data o Consensus sequences may not capture low frequency mammalian-adaptive mutations o High genetic diversity impairs identification of precursor strains o Limited reporting of negative surveillance data • Associative – Information produced is correlative, not causative

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?

Experimental Approach	Benefits	Limitations
Alt-GoF #2 [2]: Comparative sequence analysis of animal isolates from two species	• Identifies genetic traits that are associated with cross-species adaptation/transmissibility o Comparison of genetically similar viruses, such as precursor strains/spillover pairs, can result in the identification of previously unknown genetic traits that are associated with mammalian adaptation o Depending on the size of analysis and strength of association some traits can be considered "causally" linked o Analyzes **broad** data sets applicable to many strains • Gain insight into the prevalence and distribution of genetic traits o Can infer functional generalizability of genetic traits (i.e., whether they do or do not behave similarly in different genetic contexts)	• Lack of correlate[a] – Animal-origin viruses have limited capacity to infect and transmit in humans, limiting available data • Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Limited by the quality and availability of existing surveillance data o Consensus sequences may not capture low frequency mammalian-adaptive mutations o High genetic diversity impairs identification of precursor strains o Limited reporting of negative surveillance data • Associative – Information produced is correlative, not causative • Translatability – Results may not translate to adaptation in humans o Whether animals under study are representative models for human disease has not been established
Alt-GoF #3 [3]: Characterization of wild type viruses	• Identifies genetic and phenotypic traits that are associated with mammalian adaptation/transmissibility o Comparison of genetically similar viruses, such as precursor strains/spillover pairs, can result in the identification of **sufficient** genetic and phenotypic traits • Gain insight into phenotypes underlying adaptation/transmissibility	• Lack of correlate[a] – Animal-origin viruses may have limited capacity to infect animal models and have a highly limited capacity to transmit in animal models, limiting suitable isolates for this approach • Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Associative – Information produced is correlative, not causative

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?		
Experimental Approach	**Benefits**	**Limitations**
Alt-GoF #4 [4]: LoF forward genetic screen to introduce genetic changes that may attenuate transmissibility, followed by testing *in vitro* or *in vivo*	• Identifies previously unknown genetic and phenotypic traits that are necessary for mammalian adaptation/enhanced transmissibility • Gain insight into phenotypes underlying adaptation/transmissibility	• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Narrow breadth – Results may not generalize to other virus strains • Limited Range – Limited to investigation of transmissible seasonal or pandemic viruses; • Attenuated virus may recover transmissibility during characterization • Bias – Limited to investigation of previously identified *phenotypic* traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Triviality – May uncover mutations that indirectly attenuate adaptation/transmissibility, which provides limited mechanistic insight
Alt-GoF #5 [5,11,15]: Targeted LoF to introduce genetic changes expected to attenuate transmissibility	• Identifies genetic and phenotypic traits that are **necessary** for mammalian adaptation/transmissibility (i.e., provides causative data) • Gain insight into phenotypes underlying adaptation/transmissibility • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Narrow breadth – Results may not generalize to other virus strains • Limited Range – Limited to investigation of transmissible seasonal or pandemic viruses; results may not generalize to other influenza sub-types • Attenuated virus may recover transmissibility during characterization • Bias – Limited to investigation of previously identified phenotypic *or* genetic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Triviality – May uncover mutations that indirectly attenuate adaptation/transmissibility, which provides limited mechanistic insight • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?		
Experimental Approach	**Benefits**	**Limitations**
Alt-GoF #6 [6]: (*In vitro*, virus-free) Forward genetic screen to introduce genetic changes that may alter phenotypes underlying adaptation/transmissibility	• Identifies novel genetic traits that are sufficient to alter phenotypes underlying adaptation/transmissibility • Provides insight into mechanistic basis of underlying phenotypes • *In vitro* methods can be used to screen a larger number of mutants than *in vivo* methods • Proactive - can be performed using virus gene segments from viruses that do not yet transmit between humans in nature	• Simplicity of model system – Results based on the study of a viral protein/phenotype in isolation may not be recapitulated in the context of the full virus • Narrow breadth – Results may not generalize to other virus strains • Bias – Limited to investigation of previously identified phenotypic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • State of methodology – Relies upon phenotypic assays, which may be unreliable or unavailable • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved
Alt-GoF #7 [12,16]: (*In vitro*, virus-free) Targeted genetic modification to introduce genetic changes expected to alter phenotypes underlying adaptation/transmissibility	• Identifies genetic traits that are **necessary** and **sufficient** to alter a phenotype underlying adaptation/transmissibility • Provides insight into the mechanistic basis of phenotypes underlying adaptation/transmissibility • Proactive - can be performed using virus gene segments from viruses that do not yet transmit between humans in nature • Enables testing of markers in different viral gene segments to assess generalizability of previous findings	

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals		
Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?		
Experimental Approach	**Benefits**	**Limitations**
Alt-GoF #8 [7]: (*In vitro*, virus-free) Structural studies to analyze the molecular basis of adaptation/transmissibility	• Provides insight into biophysical mechanisms underlying virus-host and virus-virus protein interactions ○ Provides detailed mechanistic information • Proactive - can be performed using select virus gene segments from viruses that do not yet transmit between humans in nature depending on the state of methodology	
Alt-GoF #9 [13,17]: (*In silico*, virus-free) Modeling to analyze the biophysical effects of mutations contributing to phenotypes underlying adaptation/transmissibility	• Provides insight into biophysical mechanisms underlying virus-host and virus-virus protein interactions • Provides detailed mechanistic information • Proactive - can be performed on virus gene segments from viruses that do not yet transmit between humans in nature • Enables prediction of phenotypic consequences of markers in different viral gene segments to assess generalizability of previous findings	• Predictive – Does not confirm or correlate phenotypic effects in a biological context • Simplicity of model system – Results based on the study of a viral protein/phenotype in isolation may not be recapitulated in the context of the full virus • Model accuracy – Utility of the approach depends on the quality of existing models

Alt-GoF #10 [8][c]: Proteomic screen to identify host proteins that physically interact with viral proteins during infection	• Identifies host proteins that **may** play a role in mammalian adaptation **during infection**
	  ○ Reveals **previously unknown** host factors
	  ○ Reveals **previously unknown** host-virus interactions during infection
	• Provides insight into the role of particular virus-host interactions **during infection**
		• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans
		• Narrow breadth – Results may not generalize to other virus strains
		• Bias – Limited to investigation of previously identified phenotypic traits
		• Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty
		• Identified host protein may not be functionally relevant or may play a minor role in viral life cycle

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?

Experimental Approach	Benefits	Limitations
Alt-GoF #11 [9]: Genomic screen to identify host factors that contribute to fitness	• Proactive - can be performed using viruses that do not yet transmit between humans in nature	• Indirect – Identification of host proteins or virus-host interactions that contribute to adaptation/transmissibility provides indirect information about viral genetic and phenotypic traits underlying adaptation o Mechanistic insight may depend on prior knowledge of virus-host interactions
Alt-GoF #12 [10]: (*In vitro*, virus-free) Proteomic or genomic screen to identify host factors that interact with particular virus proteins and/or contribute to fitness	• Identifies host proteins that **may** play a role in mammalian adaptation o Reveals **previously unknown** host factors contributing to underlying phenotypes o Reveals **previously unknown** host-virus interactions contributing to underlying phenotypes • Provides insight into the role of particular virus-host interactions • Proactive - can be performed using virus gene segments from viruses that do not yet transmit between humans in nature	• Narrow breadth – Results may not generalize to other virus strains • Bias – Limited to investigation of previously identified phenotypic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Identified host protein may not be functionally relevant or may play a minor role in viral life cycle • Indirect – Identification of host proteins or virus-host interactions that contribute to adaptation/transmissibility provides indirect information about viral genetic and phenotypic traits underlying adaptation o Mechanistic insight may depend on prior knowledge of virus-host interactions • Simplicity of model system – Results based on the study of a viral protein/phenotype in isolation may not be recapitulated in the context of the full virus

Table 15.16. Summary of the Benefits of GoF Approaches That Enhance Transmission in Mammals

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Mammalian Adaptation and Transmissibility of Influenza Viruses?

Experimental Approach	Benefits	Limitations
Alt-GoF #13 [18]: Targeted modification of host factor to alter expression or function of host factors expected to contribute to adaptation/transmissibility	• Enables testing of the role of host markers in adaptation/transmissibility in the context of infection with new viral strains, to assess generalizability of previous findings	• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Narrow breadth – Results may not generalize to other virus strains • Bias – Limited to investigation of previously identified phenotypic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and adaptation/transmissibility provide further uncertainty • Identified host protein may not be functionally relevant or may play a minor role in viral life cycle • Indirect – Identification of host proteins or virus-host interactions that contribute to adaptation/transmissibility provides indirect information about viral genetic and phenotypic traits underlying adaptation • Mechanistic insight may depend on prior knowledge of virus-host interactions • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved

[a] *GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets reference the order in the landscape tables (Supplementary Information).*

[b] *Note that to date, animal-origin viruses that efficiently transmit between humans have not been discovered in nature. If available, comparative sequence and phenotypic analysis represents a viable approach for identification of genetic markers associated with human adaptation/transmissibility.*

[c] *Blue text distinguishes an approach or outcome that is associated with GoF studies that enhance mammalian adaptation but not transmissibility. Animal passaging for the purpose of animal model development is discussed in the Supplementary Information.*

Table 15.17. Crosswalk: Use of Risk Mediation Strategies for Studies on Mammalian Adaptation and Transmissibility

Scientific Knowledge Benefits – Utility and Limitations of Using Attenuated Strains for GoF Approaches That Enhance Mammalian Adaptation and Transmissibility

Virus[a]	Limitations	Experimental System[b]
High Pathogenicity Strain[c] • Animal strain • Pathogenic reassortant	N/A	*In vivo* • Mammalian adaptation and transmission studies (*the virus would likely be functional and representative of wild type conditions in vitro and in vivo*) *In vitro* • Characterization of underlying phenotypes of mammalian adaptation and transmissibility (*the virus would likely be functional and representative of wild type conditions in vitro and in vivo*)
Risk mediation Reassortant-Seasonal influenza	**Genetic Context:** • Complex phenotypes are multi-genic; results may not be recapitulated in the context of the pathogenic virus **Limited Utility:** • Precludes study of the role of animal-origin HA and NA proteins, which are critical viral factors in adaptation and transmissibility **Overlapping Phenotypes:** • Method of attenuation may alter phenotypes that contribute to adaptation/transmissibility, thus interfering with the study of adaptation/transmissibility **Altered Course of Disease:** • Animals infected with attenuated viruses may exhibit significantly different disease pathology, limiting the relevance to wild type viruses	*In vivo* • Mammalian adaptation and transmission studies (*the virus may not be functional or representative in vitro or in vivo*) *In vitro* • Characterization of underlying phenotypes of mammalian adaptation and transmissibility (*the virus may not be functional or representative in vitro or in vivo*)

Table 15.17. Crosswalk: Use of Risk Mediation Strategies for Studies on Mammalian Adaptation and Transmissibility

Scientific Knowledge Benefits – Utility and Limitations of Using Attenuated Strains for GoF Approaches That Enhance Mammalian Adaptation and Transmissibility

Virus[a]	Limitations	Experimental System[b]
Risk mediation Reassortant-Lab-adapted (e.g., PR8)	**Limited model systems:** • Lab-adapted strains are not transmissible in ferrets **Genetic Context:** • Complex phenotypes are multi-genic; results may not be recapitulated in the context of the pathogenic virus **Overlapping Phenotypes:** • Method of attenuation may alter phenotypes that contribute to adaptation/transmissibility, thus interfering with the study of adaptation/transmissibility **Altered Course of Disease:** • Animals infected with attenuated viruses may exhibit significantly different disease pathology, limiting the relevance to wild type viruses	*In vivo* • Mammalian adaptation and transmission studies (*the virus may not be functional or representative in vitro or in vivo*) *In vitro* • Characterization of underlying phenotypes of mammalian adaptation and transmissibility (*the virus may not be functional or representative in vitro or in vivo*)
Attenuated Strain • Targeted mutagenesis to remove virulence factor (e.g., ΔMBCS)	**Genetic Context:** • Complex phenotypes are multi-genic; results may not be recapitulated in the context of the pathogenic virus **Overlapping Phenotypes:** • Method of attenuation may alter phenotypes that contribute to adaptation/transmissibility, thus interfering with the study of adaptation/transmissibility **Altered Course of Disease:** • Animals infected with attenuated viruses may exhibit significantly different disease pathology, limiting the relevance to wild type viruses	*In vivo* • Mammalian adaptation and transmission studies (*the virus would likely be non-functional in vitro or in vivo*) *In vitro* • Characterization of underlying phenotypes of mammalian adaptation and transmissibility (*the virus would likely be functional and representative of wild type conditions in vitro and in vivo*)

Table 15.17. Crosswalk: Use of Risk Mediation Strategies for Studies on Mammalian Adaptation and Transmissibility

Scientific Knowledge Benefits – Utility and Limitations of Using Attenuated Strains for GoF Approaches That Enhance Mammalian Adaptation and Transmissibility

Virus[a]	Limitations	Experimental System[b]
Molecular Biocontainment • Incorporation of binding sites for miRNAs expressed in humans but not experimental animals	**Limited model systems:** • Engineered strains to date are capable of replicating in ferrets but not mice or humans, which limits the model systems that can be used for *in vivo* and *in vitro* studies [d] • Strategy has been validated in two strains only **Potential for Altered Virus Function** • Whether incorporation of miRNA target sites alters the biology of the virus, including viral pathogenesis, has not yet been extensively characterized	*In vivo* • Mammalian adaptation and transmission studies in ferrets[c] (*the virus would likely be functional and representative of wild type conditions in vitro and in vivo*) *In vitro* • Characterization of underlying phenotypes of mammalian adaptation and transmissibility using cells that do not express miR-192 (excludes human cell lines)[c] (*the virus may not be functional or representative in vitro or in vivo*)

[a] *Animal-origin strains include avian- and swine-origin strains that have and have not infected humans. Pandemic strains include the 1918 H1N1, 1957 H2N2, and 1968 H3N2 viruses. Seasonal strains include all seasonal isolates and 2009 H1N1 pandemic isolates (now circulating seasonally). Risk mediation reassortants include all reassortants with lab-adapted viruses or with surface protein gene segments from seasonal influenza viruses. Pathogenic reassortants include viruses with animal and/or human gene segments (both seasonal and pandemic) for which human populations have limited or no immunity.*

[b] *The text color in the experimental system column indicates the general feasibility of the use of the virus described for in vivo or in vitro use. Green indicates that the virus would likely be functional and representative of wild type conditions in vitro and in vivo, orange indicates that the virus may not be functional or representative in vitro or in vivo, and red indicates that the virus would likely be non-functional in vitro or in vivo.*

[c] *GoF approaches are shaded in blue, and alt-GoF approaches (i.e., conducting GoF approaches using attenuated strains in lieu of wild type strains) are shaded in grey.*

[d] *Langlois et al. incorporated target sites for miR-192, which is expressed in humans and mice but not ferrets, into the HA genome segment of two different influenza A strains, thereby generating an engineered strain that is replication-competent in ferrets but not humans or mice.*[1429]

[e] *Assessment of suitable experimental systems reflects miRNA-based molecular biocontainment strategies published to date, i.e., the use of miR-192 target sites by Langlois et al.*

[1429] Langlois RA *et al* (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844-847

15.3.4 Benefits to Surveillance

Influenza pandemics occur when a novel influenza virus becomes transmissible in human populations with limited pre-existing immunity. The likelihood and potential consequences of a pandemic are the result of complex interactions between multiple factors related to the properties of the virus, of the host population, and of the environment in which the virus is circulating.[1430] Analysis of the phenotypic properties of individual surveillance isolates informs the components of pandemic risk assessments related to the properties of the virus. This section focuses on GoF benefits to these surveillance efforts. Ultimately, GoF benefits to surveillance improve the health of human populations through public health activities undertaken subsequent to a pandemic risk assessment, such as development of pre-pandemic vaccines. Thus, the scope of GoF benefits to surveillance depends on the value of GoF data relative to other factors that are considered in the risk assessment process. The process of pandemic risk assessment, including descriptions of other factors that are considered in risk assessments as well as downstream decision-making about pandemic preparedness policies is described in detail in 15.3.5.

Influenza surveillance is conducted in human and animal populations, including agricultural animals, companion animals, and wildlife. The WHO Global Influenza Surveillance and Response System (GISRS) serves as a central repository for data about animal influenza infections in humans, generated through passive surveillance (i.e., reporting of illnesses in patients who interact with the healthcare system).[1431] The GISRS is a two-tiered system, structured such that clinical samples from patients are initially collected by National Influenza Centers (NICs) located throughout the world, which perform preliminary diagnostic tests and forward samples with evidence of animal influenza infection to WHO Collaborating Centres (WHOCCs) for thorough characterization.[1432] In contrast, animal surveillance is generally ad hoc, reflecting a combination of passive surveillance and active sampling of agricultural animals or wildlife, and data are spread throughout several different surveillance systems. Collectively, the goal of this surveillance is to monitor the evolution of circulating animal influenza viruses, in order to identify those viruses that pose a risk of emerging in human populations to cause a pandemic. Resources can then be dedicated to mitigating the risk factors associated with virus emergence, for example through community-level interventions at the animal-human interface, and to prepare for a potential emergence event, for example through the development of pre-pandemic vaccines.[1433]

Multiple virus properties contribute to the likelihood that the virus will adapt to efficiently transmit in human populations and the potential consequences of that event:

- Whether the virus is adapted (or poised to adapt) to efficiently infect and transmit between humans,
- Viral virulence,
- Whether the strain is antigenically similar to existing candidate vaccine viruses and stockpiled pre-pandemic vaccines, and

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1430 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

1431 The World Health Organization. Global Influenza Surveillance and Response System (GISRS). http://www.who.int/influenza/gisrs_laboratory/en/. Last Update November 2, 2015. Accessed November 6, 2015.

1432 There are six WHOCCs, including the U.S. Centers for Disease Control in Atlanta, GA and St. Jude's Children's Research Hospital in Memphis, TN.

1433 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

- Whether the virus is sensitive to existing antivirals.

Each of these properties can be directly measured in the laboratory or can be inferred from the genetic sequence based on the presence of molecular markers that have been linked to those phenotypes through previous research. In practice, due to the limitations of both strategies, the strategies are utilized together. Two other approaches are in development but are not yet used in public health practice. The first involves the use of rapid assays to assess phenotypes underlying mammalian adaptation, transmissibility, and virulence (i.e., versus evaluating the complex phenotype through animal experiments). The second involves computational modeling to predict phenotype from genotype, which incorporates experimental data about mutations that give rise to phenotypic changes, structural data, and other types of data. This section analyzes how GoF research can improve strategies for evaluating mammalian adaptation, transmissibility, and virulence, including strategies that are currently used and those that are in development. The role of GoF in surveillance for antiviral resistance is evaluated in Section 16.6. First, the utility and limitations of traditional methods for laboratory evaluation of the infectivity, transmissibility, and virulence of surveillance viruses are evaluated. This information motivates the need for development of additional approaches that can provide information about these virus properties, the quality of which can be improved by GoF approaches.

The pathogenicity and the ability of an animal influenza virus to infect and transmit in mammals is typically evaluated in ferrets, though mice may also be used for pathogenicity testing.[1434] The strength of these assays is that they directly measure the complex properties of mammalian adaptation, transmissibility, and virulence. However, multiple shortcomings are associated with reliance on these assays for evaluating the transmissibility and virulence of animal flu viruses collected through surveillance. First, these assays are unable to assess when viruses have acquired underlying properties that are necessary but not sufficient to enhance infectivity, transmissibility, or virulence, and such knowledge about partial adaptation is of interest for pandemic risk assessments. Second, these assays require the use of surveillance isolates, which limits the number of viruses that can be subjected to phenotypic characterization. Although in principle, viruses can be synthetically reconstructed based on published sequences, in practice the publicly available sequence information is often incomplete.[1435] Third, viruses may acquire mutations that alter their properties during isolation in eggs or cells, in which case the results of the phenotypic assay will not reflect the properties of the virus present in the original clinical sample. Fourth, transmission and virulence testing in animals requires technical expertise and must be conducted under BSL-3 conditions, limiting the conduct of these assays to the six WHOCCs (which include the Centers for Disease Control in Atlanta, GA and St. Jude Children's Research Hospital in Memphis, TN).[1436,1437] Finally, when viruses of concern are initially detected abroad, political and regulatory factors may delay the shipping of the isolate to US labs, thereby delaying the generation of phenotypic data. Although the WHO Pandemic Influenza Preparedness Framework calls for NICs to ship clinical specimens and/or viruses that cannot be readily identified to a WHOCC or a H5 Reference Laboratory within one week, delays arising from political and logistical factors still occur.[1438,1439] US select agent regulations also considerably delay the receipt of highly pathogenic avian influenza viruses in US labs. One governmental official involved in the pandemic risk assessment process estimated that the time

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[1434] (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.
[1435] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136
[1436] In some cases, transmissibility and virulence testing in ferrets may be conducted by university or other diagnostic labs that have collaborative relationships with NICs.
[1437] (2015a) Interview with U.S. CDC (Centers for Disease Control) representative
[1438] (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments
[1439] The World Health Organization. (2011b) Pandemic influenza preparedness framework for the sharing of influenza viruses and access to vaccines and other benefits. pp. 1-67.

needed to work through regulatory logistics delays receipt of HPAI samples by three to four weeks, which may be compounded by political or logistical issues on the part of the sending country.[1440]

For the reasons listed above, the CDC has incorporated the use of molecular markers for phenotypes of concern into the pandemic risk assessment process to complement data from animal models. Because the phenotypes of mammalian adaptation, transmissibility, and virulence are complex, arising from the interplay between multiple underlying phenotypes, this strategy involves inspecting sequences for markers that are casually linked to underlying phenotypes (e.g., altered sialic acid receptor binding specificity). Sequences may be inspected for the presence of particular mutations or for the presence of substitutions at particular amino acid positions. In the latter case, structural analysis and molecular modeling may be used to predict whether the substitutions has the same phenotypic effect as other validated substitutions at that site. Because a constellation of amino acid changes is needed for an animal virus to evolve to efficiently infect, transmit, and cause disease in people, molecular markers are considered collectively to determine the overall risk associated with a virus. Importantly, this process assumes that the complex phenotypes of mammalian adaptation, transmissibility, and virulence can accrue in a step-wise fashion, such that "partially adapted" viruses can persist in nature. (If true, the ability to detect "partially adapted" viruses that are poised for emergence in human populations is a strength of reliance on molecular marker data, as partial phenotypes may not be detected using phenotypic assays for mammalian adaptation, transmissibility, and virulence.)

Influenza research experts agree that the state of this science does *not* enable accurate and reliable prediction of phenotype from genotype for complex phenotypes such as mammalian adaptation, transmissibility, and virulence. Multiple sources of scientific uncertainty limit current capabilities, which can be broadly grouped into two categories: (1) uncertainties related to the phenotypes underlying adaptation, transmissibility, and virulence and (2) uncertainties related to the genetic traits that alter underlying phenotypes.

Uncertainties Related to Phenotypes Underlying Mammalian Adaptation, Transmissibility, and Virulence:

- Weak linkage between underlying phenotypes and adaptation/transmissibility/virulence – that is, uncertainty in whether particular underlying phenotypes, such as altered sialic acid receptor binding specificity, are necessary for complex phenotypes, such as mammalian adaptation across many different virus strains.

- Lack of knowledge about how underlying phenotypes interact to alter adaptation, transmissibility, and virulence (i.e., how to integrate the presence of multiple markers to appropriately determine overall risk).

- Lack of knowledge about whether complex phenotypes can slowly accrue (i.e., whether partially adapted viruses can persist in nature) or whether the acquisition of efficient infectivity, transmissibility, and enhanced virulence in mammals is an "all-or-none" phenomenon.

Uncertainties Related to the Genetic Traits That Alter Underlying Phenotypes

- Inability to predict whether a particular amino acid substitution identified in one strain will have similar phenotypic consequences in other strains.

- Lack of knowledge about whether different amino acid substitutions at a particular amino acid position will have similar phenotypic consequences as known mutations;

[1440] (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.

- Lack of knowledge about the mutational landscape that permits evolution of a complex phenotype – e.g., how many different sets of mutations enable the acquisition of airborne transmissibility?

Collectively, these sources of uncertainty significantly compromise the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence. However, the state of the science supporting individual markers varies widely. The phenotypic consequences of certain markers, such as the E627K mutation in the PB2 gene which lowers the optimal temperature for polymerase activity, have been shown to be conserved in the context of multiple virus strains, and this marker has also been shown to be enriched in human isolates of H5N1 relative to avian isolates.[1441, 1442, 1443, 1444] (Notably, this mutation was absent from the 2009 H1N1 pandemic virus, highlighting the point that multiple evolutionary pathways permit adaptation of animal influenza viruses to humans.[1445, 1446]) However, most markers are not well-validated, either because their function is not conserved or not yet been tested across multiple strain contexts.

Given the shortcomings associated with phenotypic assays and molecular marker data, the use of computational methods for sequence-based predictions of phenotypes underlying mammalian adaptation, transmissibility, and virulence has also been proposed.[1447] Although a variety of computational methods have shown promise for predicting phenotype from genotype, for those "known" phenotypes associated with adaptation/transmissibility, the accuracy of their predictions remains largely unknown.[1448, 1449]

GoF approaches have potential to address shortcomings associated with the use of virological data, molecular markers, and computational methods to evaluate the infectivity, transmissibility, and virulence of animal influenza viruses in mammals, representing three different strategies for improving upon the status quo. The value of each strategy and the utility and limitations of GoF approaches for improving each strategy, relative to alt-GoF approaches, are discussed below.

15.3.4.1 Analysis of GoF and Alt-GoF Approaches That Support the Development of Rapid Phenotypic Assays

GoF approaches that identify new phenotypes underlying mammalian adaptation, transmissibility, and virulence, that strengthen the linkage between underlying phenotypes and complex phenotypes, and that provide insight into how underlying phenotypes synergize to alter host tropism, transmissibility, and virulence provide a foundation for the development of rapid phenotypic assays.

1441 Qi L *et al* (2014) Contemporary Avian Influenza A Virus Subtype H1, H6, H7, H10, and H15 Hemagglutinin Genes Encode a Mammalian Virulence Factor Similar to the 1918 Pandemic Virus H1 Hemagglutinin. *mBio* 5: e02116-02114

1442 Steel J *et al* (2009) Transmission of Influenza Virus in a Mammalian Host Is Increased by PB2 Amino Acids 627K or 627E/701N. *PLoS pathogens* 5: e1000252

1443 Le QM *et al* (2009) Selection of H5N1 influenza virus PB2 during replication in humans. *Journal of virology* 83: 5278-5281

1444 Luk GS *et al* (2015) Transmission of H7N9 Influenza Viruses with a Polymorphism at PB2 Residue 627 in Chickens and Ferrets. Ibid. 89: 9939-9951

1445 Bussey KA *et al* (2010) PB2 residue 271 plays a key role in enhanced polymerase activity of influenza A viruses in mammalian host cells. Jbid. 84: 4395-4406

1446 Herfst S *et al* ibid.Introduction of virulence markers in PB2 of pandemic swine-origin influenza virus does not result in enhanced virulence or transmission. 3752-3758

1447 Russell CA *et al* (2014) Improving pandemic influenza risk assessment. *Elife* 3: e03883

1448 (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.

1449 Russell CA *et al* (2014) Improving pandemic influenza risk assessment. *Elife* 3: e03883

15.3.4.1.1 Strengths and Weaknesses of Using Rapid Phenotypic Assays to Inform Pandemic Risk Assessments

Rapid assays to measure phenotypes underlying mammalian adaptation, transmissibility, and virulence could be performed in lieu of traditional evaluation of these complex phenotypes using ferrets. The development of rapid phenotypic assays holds promise for improving analysis of surveillance data for several reasons. First, the use of assays that are higher throughput than ferret testing will enable the phenotypic characterization of a larger number of viruses. Second, for those assays interrogating the function of a single protein or a protein complex, synthesizing the relevant genes based on publicly available genetic sequence data may be feasible, which would enable the characterization of viruses for which isolates are not available. In the event that *in vitro*, virus-free rapid phenotypic assays can be developed, these assays would pose lower lab safety risks than ferret testing using full, infectious virus. Third, rapid phenotypic assays that require less technical expertise than ferret experiments are better suited for NICs, which would shorten the time lag between the initial detection and phenotypic characterization of a given virus. Thus, taken together, the development of rapid phenotypic assays has the potential to expand the quantity and the timeliness of phenotypic characterization data available for pandemic risk assessments. However, these assays will need to be carried out under BSL-3 conditions, which will limit the number of diagnostic laboratories that will be able to conduct the assays. Notably, the majority of NICs do not have BSL-3 capabilities, particularly in countries in which animal influenza viruses of concern are circulating (as BSL-3 capabilities are not needed for isolation of seasonal influenza viruses, which comprises the bulk of the diagnostic workload of NICs). That said, the number of NICs with BSL-3 capabilities themselves (or with access to BSL-3 labs through collaborative relationships with university labs, US military labs such as NAMRU-3, or other labs) has increased since 2005 and is likely to continue to increase.[1450,1451] Though challenging due to the expense and technical expertise needed to construct and run a BSL-3 lab, this increase will facilitate the conduct of rapid phenotypic assays using whole viruses in the future.

In order for rapid phenotypic assays to be useful as proxies for mammalian adaptation, transmissibility, and virulence, the measured phenotype must be strongly linked to adaptation/transmissibility/virulence across many strain contexts. Additionally, interpretation of the results requires knowledge about how individual phenotypes contribute to overall pandemic risk, which relies on an understanding of how underlying phenotypes synergize to shape complex phenotypes. Gaps in scientific knowledge related to the phenotypes underlying mammalian adaptation, transmissibility, and virulence, described above, constrain the development and use of rapid phenotypic assays. As discussed in detail in Sections 15.3.3.3 and 15.4.3.1, both GoF and alt-GoF approaches can provide insight into these scientific questions. The relevant findings are summarized below.

15.3.4.1.2 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

GoF approaches represent the most efficient and effective approach for identifying novel *phenotypic* traits underlying mammalian adaptation, transmissibility, and virulence. Critically, GoF approaches are uniquely capable of discovering phenotypic traits underlying the transmissibility of animal influenza viruses because these viruses do not efficiently transmit between humans in nature. Furthermore, targeted genetic modification of viruses to introduce genetic traits that alter underlying phenotypes is uniquely capable of demonstrating that a particular phenotype is causally linked to enhanced infectivity/transmissibility/ virulence in mammals across multiple virus contexts. Additionally, the ability to alter phenotypes individually and in combination (i.e., through incorporation of varying sets of

[1450] (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.
[1451] Navy Medical Research Center. Naval Medical Research Unit 3 (NAMRU-3) Cairo, Egypt. http://www.med.navy.mil/sites/nmrc/Pages/namru3.htm. Last Update Accessed November 28, 2015.

mutations) provides insight into how multiple underlying phenotypes interact to enhance infectivity, transmissibility, or virulence in mammals. This approach can also determine how an "intermediate" level of adaptation/transmissibility/virulence (i.e., acquisition of some but not all phenotypic traits that are required for viruses to efficiently infect) causes disease, transmits in mammals, and affects viral fitness, which may provide insight into whether such partially adapted strains can persist in nature. However, the major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to human disease in nature.

Alternative approaches have significant limitations relative to GoF approaches. Characterization of wild type viruses provides limited insight into phenotypic traits underlying mammalian adaptation and transmissibility because animal influenza viruses that efficiently infect and transmit in humans do not exist in nature. However, characterizing the constellation of underlying phenotypes present in a large number of wild type viruses (e.g., sialic acid receptor binding specificity, HA stability, optimal temperature for polymerase activity, etc.) is uniquely capable of providing insight into whether viruses that have a subset of the properties that are necessary for enhanced infectivity, transmissibility, or virulence can persist in nature.

LoF approaches have limited utility for broad and unbiased identification of phenotypic traits that contribute to transmissibility and pathogenicity due to their inefficiency, as a limited number of mutants can been screened through ferret transmission studies due to technical and ethical concerns and mutants may recover transmissibility during the single round of infection needed for characterization. An additional limitation is that the fact that mechanisms underlying transmissibility of seasonal/pandemic viruses may not translate to animal influenza viruses. Though LoF approaches can be used to causally demonstrate that a particular phenotype is necessary for efficient transmissibility and enhanced virulence, this approach cannot be used to understand how multiple phenotypes synergize to enhance infectivity, transmissibility, or virulence. This information critically informs how results from multiple phenotypic assays should be integrated to evaluate overall pandemic potential. Surveillance-based approaches, including comparison of human and animal isolates, comparison of sequences spanning avian to mammalian adaptation events, and comparison of viral isolates with varying levels of virulence are limited to the study of previously known traits and provide associative data. Notable exceptions include the analysis of precursor/spillover pairs for the study of adaptation/transmissibility and analysis of viral isolates over the course of infection in a single patient for the study of virulence. However, the availability of both types of paired isolates is low. In addition, neither surveillance-based approaches nor LoF approaches can provide insight into phenotypes underlying transmissibility because animal influenza viruses that efficiently transmit in humans do not exist in nature. *In vitro*, virus free approaches, which involve the study of known phenotypes in isolation, cannot provide information about the functional relationships among underlying phenotypes or between underlying phenotypes and adaptation/transmissibility.

15.3.4.2 Analysis of GoF and Alt-GoF Approaches That Support the Use of Molecular Markers to Evaluate the Risk Posed by Circulating Animal Influenza Viruses

GoF approaches support the use of molecular marker data to evaluate the risk posed by circulating animal influenza viruses in two ways: (1) through the discovery of novel molecular markers of phenotypic properties of concern and (2) by strengthening the predictive value of known molecular markers.

15.3.4.2.1 Strengths and Weaknesses of Using Molecular Marker Data to Inform Pandemic Risk Assessments

The use of molecular marker data to evaluate the pandemic potential of animal influenza viruses has several strengths relative to the use of phenotypic data. In particular, the fact that clinical isolates can be

directly sequenced provides several advantages. First, direct sequencing of clinical isolates avoids the problem that the composition and properties of viral species present in the clinical sample could change during the virus isolation process. Second, following inactivation of virus present in a clinical sample, the sequencing procedure can be carried out under BSL-2 conditions and thus can more feasibly be implemented at NICs and other diagnostic labs in developing countries. Third, whether from clinical samples or virus isolates, sequencing is becoming ever cheaper and easier. As a result, viral genetic sequence data is currently the fastest and most reliable data generated by diagnostic labs in areas where viruses of concern are circulating.[1452] However, most genetic surveillance data is generated by sequencing the HA and NA genes of viral isolates at WHOCCs.[1453] Full realization of the benefits that can be derived from the use of molecular marker data will require expanding the sequencing capabilities of diagnostic laboratories that comprise the "base" of the influenza surveillance system as well as increasing the proportion of clinical samples that are directly sequenced. Additionally, the number of viruses that are subjected to whole genome sequencing (as opposed to sequencing the HA and NA genes only) must be increased in order to fully utilize molecular markers in genes other than HA and NA. Notably, stakeholders throughout the surveillance system recognize that capabilities in each of these areas – sequencing at NICs, direct sequencing of clinical samples, and whole genome sequencing – are desirable and are striving to implement them whenever and wherever possible.[1454]

As described above, the current utility of molecular markers to the interpretation of genetic surveillance data is constrained by multiple sources of scientific uncertainty. Additionally, as knowledge about the phenotypes underlying mammalian adaptation, transmissibility, and virulence is incomplete, the discovery of additional molecular markers associated with novel underlying phenotypes would broaden the utility of this approach. As discussed in detail in Sections 15.3.3.3 and 15.4.3.1, both GoF and alt-GoF approaches can provide insight into these scientific questions. The relevant findings are summarized below.

15.3.4.2.2 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The benefits of GoF approaches relative to alt-GoF approaches for addressing knowledge gaps at the phenotypic level were summarized in Section 15.3.4.1.2. In brief, GoF approaches represent the most efficient and effective methods for discovering novel phenotypes underlying adaptation/transmissibility/virulence and are uniquely capable of demonstrating that phenotypes are causally linked to enhanced infectivity/transmissibility/virulence of animal influenza viruses in representative animal models. GoF approaches are also uniquely capable of providing definitive information about how multiple phenotypes synergize to promote mammalian adaptation, efficient transmissibility, and virulence. However, alt-GoF approaches, namely characterization of wild type viruses, are uniquely capable of demonstrating whether partially adapted viruses exist in nature, which provides insight into whether complex phenotypes such as adaptation, transmissibility, and virulence can accrue in a step-wise fashion (an underlying assumption of the use of molecular markers to evaluate pandemic risk).

Both GoF and alt-GoF approaches can provide insight into the scientific knowledge gaps related to the *genetic* traits underlying mammalian adaptation, transmissibility, and virulence. GoF approaches represent the most efficient and effective approach for identifying novel genetic traits underlying mammalian adaptation, transmissibility, and virulence. Furthermore, targeted genetic modification of viruses to introduce genetic traits associated with mammalian adaptation/transmissibility/virulence is uniquely capable of demonstrating that particular genetic markers are *necessary* and *sufficient* for mammalian adaptation, transmissibility, or enhanced virulence across multiple virus contexts. In addition,

[1452] (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.
[1453] Ibid.
[1454] Ibid.

GoF approaches, namely forward genetic screens, are uniquely capable of systematically exploring alternative mutational pathways for altering an underlying phenotype (e.g., changing sialic acid receptor binding specificity) in the context of whole virus. The major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to the potential to cause a pandemic in humans.

Alternative approaches have significant limitations relative to GoF approaches. Characterization of wild type viruses provides limited insight into genetic traits underlying mammalian adaptation and transmissibility because animal influenza viruses that efficiently infect and transmit in humans do not exist in nature. LoF approaches have limited utility for broad and unbiased identification of novel genetic traits that are necessary for transmissibility or enhanced virulence due to their inefficiency and the fact that mechanisms underlying transmissibility of seasonal/pandemic viruses may not translate to animal influenza viruses. Surveillance-based approaches, including comparison of human and animal isolates and of sequences spanning avian to mammalian adaptation events, have limited utility for the discovery of *novel* genetic traits associated with adaptation/transmissibility/virulence due to the high genetic diversity of influenza viruses and shortcomings in the quality and availability of surveillance data. A notable exception is the comparison of genetically similar viruses such as precursor/spillover strains and the comparison of viral isolates over the course of illness in a single patient, though such paired isolates are rarely available. However, surveillance-based approaches have several unique strengths for validating the functional consequences of particular markers. Comparison of human and animal isolates or of human isolates with varying levels of virulence is uniquely capable of providing direct insight into traits associated with human adaptation and virulence across multiple strain contexts. These traits can be considered "causally" linked if a large enough number of sequences are compared. Notably, this approach cannot be used to validate markers associated with enhanced transmissibility because animal influenza strains that transmit efficiently between humans do not exist in nature. The high-throughput nature of *in vitro*, virus free approaches relative to animal experiments renders them appealing for the discovery of additional mutations that give rise to particular phenotypic changes (through forward genetic screens) and for validating the function of particular markers in new genetic contexts. However, results may not be recapitulated *in vivo*, in the context of the full virus.

Notably, the feasibility of using molecular markers to infer phenotype from genotype depends on several factors: (1) the extent to which the functional consequences of particular markers are conserved across multiple strain contexts, (2) the number of different sets of mutations that give rise to a phenotype of interest, and (3) whether the phenotypic changes associated with adaptation/transmissibility/virulence arise due to the concerted effects of many mutations, each of which has a small individual effect, or whether single mutations give rise to large phenotypic changes. Influenza researchers emphasized that for the known phenotypes associated with adaptation, transmissibility, and virulence, the answers to these questions are unknown and are likely to vary by phenotype. For example, it is likely that a large number of distinct mutations are capable of increasing HA stability and that the set of mutations that increase HA stability will vary by strain. Thus, this phenotype may not be a good candidate for the molecular marker approach, but rather for the rapid phenotypic assay approach. Several researchers felt that performing a limited number of GoF experiments to address each of these three questions would enable the determination of whether delineating the set of mutations that can give rise to a particular phenotype is achievable through a reasonable number of experiments.

15.3.4.3 Analysis of GoF and Alt-GoF approaches That Improve Predictive Models

GoF experiments that provide data about whether particular mutations alter phenotypes of concern have potential to improve existing computation models for predicting phenotype from genotype.

15.3.4.3.1 Strengths and Weaknesses of Using Computational Models to Inform Pandemic Risk Assessments

As the use of computational models to predict phenotypes underlying mammalian adaptation, transmissibility, and virulence capitalizes on (and depends on) the availability of sequence data, the strengths and limitations of this approach relative to the use of virologic data are similar to those described above for the use of molecular marker data.

Existing computational models cannot reliably predict phenotypes underlying mammalian adaptation, transmissibility, and virulence based on sequence information. Additional experimental data is needed to appropriately parameterize models, and experiments must be conducted to validate the phenotypic predictions of models. Both GoF and alt-GoF approaches can generate data that improves the accuracy of existing models.

15.3.4.3.2 Summary - Benefits of GoF Approaches Relative to Alt-GoF Approaches

A variety of experimental data are needed to improve the accuracy of existing models, including data about mutations that do and do not give rise to phenotypic changes of interest. These data are critical for building models that can account for the context dependence of genetic changes in influenza biology. GoF approaches (targeted mutagenesis and forward genetic screens) are uniquely capable of generating these data in the context of the full virus, although *in vitro*, virus free approaches can also be used.

In contrast, additional experimental data about the biophysical basis of underlying phenotypes, such as crystallography data and measurements of HA binding affinities to α2,6 and α2,3 sialoglycans, is also needed to improve existing models. These data are generated through alternative experimental approaches.

Finally, model predictions must be validated experimentally, and results feedback to improve model accuracy. While predictions can be tested using *in vitro*, virus free assays, experimental validation in the context of the full virus (GoF) is also important.

Taken together, GoF and alt-GoF approaches provide different types of experimental data that are both essential for improving the accuracy of predictive models, and GoF approaches are uniquely capable of validating model predictions in the context of the full virus.

15.3.4.4 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches to Surveillance

A key goal of influenza surveillance is to monitor the evolution of circulating animal influenza viruses, in order to identify those viruses that pose a risk of emerging in human populations to cause a pandemic. Resources can then be dedicated to mitigating the risks of an emergence event. Analysis of the phenotypic properties of individual surveillance isolates is an important aspect of pandemic risk assessments, including transmissibility and virulence in mammals. Currently, this analysis relies on the laboratory characterization of surveillance isolates and, to a lesser extent, the inspection of sequences for molecular markers associated with phenotypes underlying mammalian adaptation, transmissibility and virulence. Both methods exhibit shortcomings that compromise the accuracy, timeliness, and quantity of data. Two additional approaches are in development to address these shortcomings: rapid assays for phenotypes underlying mammalian adaptation and transmissibility and computational models to predict underlying phenotypes from genotype. Such rapid phenotypic assays do not yet exist, and the prospective accuracy of existing models is unknown. Both GoF and alt-GoF experimental approaches have potential to address shortcomings associated with the use of rapid phenotypic assays, molecular markers, and computational models.

GoF approaches provide unique benefits to the design and validation of rapid assays for phenotypes underlying adaptation, transmissibility, and virulence. The fact that these assays would be high-throughput, less technically challenging than ferret experiments and could likely utilize synthetically generated viral gene segments could increase the quantity and timeliness of phenotypic data available, relative to the use of traditional phenotypic characterization assays for adaptation, transmissibility, and virulence. The accuracy and utility of rapid phenotypic assays depends on establishing a strong linkage between underlying phenotypes and adaptation/transmissibility/virulence as well as developing an understanding of how multiple phenotypes synergize to enhance the infectivity, transmissibility, and virulence of animal influenza viruses in mammals. GoF approaches represent the most efficient and effective approach for discovering novel phenotypes underlying mammalian adaptation, transmissibility, and virulence and are uniquely capable of demonstrating that a particular phenotype is causally linked to enhanced infectivity/transmissibility/virulence in mammals across multiple virus contexts. GoF approaches are also uniquely capable of causally determining how multiple underlying phenotypes interact to enhance infectivity, transmissibility, or virulence in mammals, which provides insight into how information about underlying phenotypes should be integrated for a risk assessment. However, a major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to human disease in nature. Characterizing the constellation of underlying phenotypes present in a large number of wild type viruses (alt-GoF) is uniquely capable of providing insight into whether partially adapted viruses can persist in nature, which lends support to the practice of inferring complex phenotypes such as adaptation, transmissibility, and virulence based on data about underlying phenotypes. Ultimately, the utility of these assays depends on whether phenotypes underlying mammalian adaptation, transmissibility, and virulence are conserved across different strains, which is not yet well-understood. However, the fact that the same underlying phenotypes were shown to confer airborne transmissibility to two very different H5N1 strains – a fully avian clade 2.1 H5N1 strain and an H5N1 reassortant strain containing an avian clade 1 H5 gene and the remaining genes from a 2009 H1N1 pandemic virus – suggests that conserved mechanisms may exist.[1455,1456] Finally, a notable barrier to realization of the benefits derived from the use of rapid phenotypic assays is that these assays must be carried out under BSL-3 conditions, which limits the number of diagnostic laboratories that will be able to conduct the assays. Most NICs do not have BSL-3 capabilities, though the number of NICs with BSL-3 labs is increasing.[1457]

GoF approaches provide unique benefits to the practice of using molecular markers to infer phenotypes underlying adaptation/transmissibility/virulence based on genetic sequence data. As sequencing has become cheaper and easier, sequence data has become the fastest and most reliable type of surveillance data produced by diagnostic labs located in countries in which animal influenza viruses of concern are circulating. Furthermore, the increasing reliance on direct sequencing of clinical samples has potential to increase the accuracy of phenotypic characterization information, relative to sole reliance on traditional phenotypic assays using viral isolates. Currently, most molecular markers for mammalian adaptation, transmissibility, and virulence have low predictive value due to significant scientific uncertainties regarding the association between underlying phenotypes and adaptation/transmissibility/virulence, whether the function of markers is conserved across different strain contexts, and the breadth of mutations that can give rise to a particular phenotypic change. Additionally, it is likely that as-yet-undiscovered genetic and phenotypic traits contribute to mammalian adaptation, transmissibility, and virulence. As discussed above, GoF approaches provide essential data for strengthening the linkage between underlying phenotypes and adaptation/transmissibility/virulence. GoF approaches also provide unique advantages for

[1455] Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541
[1456] Imai M *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486: 420-428
[1457] (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

discovering novel markers and strengthening the predictive value of known markers. Namely, GoF approaches represent the most efficient and effective approach for discovering novel genetic traits underlying mammalian adaptation, transmissibility, and virulence and are uniquely capable of demonstrating that particular genetic markers are *necessary* and *sufficient* for mammalian adaptation, transmissibility, or virulence across multiple virus contexts. However, the validation of molecular markers for mammalian adaptation or virulence through analysis of genetic surveillance data (alt-GoF) is uniquely capable of providing direct insight into traits associated with *human* adaptation/virulence across multiple strain contexts, which complements GoF approaches. Notably, surveillance-based approaches are not viable for the validation of molecular markers associated with transmissibility because animal influenza strains that transmit efficiently between humans in nature do not exist. GoF approaches are also uniquely capable of systematically exploring alternative mutational pathways for modifying an underlying phenotype in the context of whole virus. *In vitro*, virus free approaches can also be used, but results may not be recapitulated in the context of the full virus. As above, the major caveat associated with GoF approaches is that results gleaned from laboratory studies involving animal models may not translate to human disease in nature. Critically, the feasibility of using molecular markers to infer phenotype from genotype will depend on the functional generalizability of particular markers, the breadth of the mutational landscape for a particular phenotypic change, and the extent to which individual mutations alter a particular phenotype. The answers to these questions are unknown and are likely to vary by phenotype. A limited number of GoF experiments will enable researchers to determine whether delineating the set of mutations that can give rise to a particular phenotype is achievable through a reasonable number of experiments. Finally, molecular markers that confer large phenotypic changes are much more useful than molecular markers that minimally modify a phenotype of interest, as integrating many mutations that each have small individual effects is likely to be difficult. To date, some markers have been found to confer substantial phenotypic changes while others have minor effects, and future discoveries are likely to be similarly mixed. Finally, notable barriers to the full realization of benefits derived from the use of molecular markers include the need to further increase the number of sequences generated at NICs, the number of clinical samples that are directly sequenced, and the number of viruses that are subjected to whole genome sequencing.

GoF approaches are also critical for improving models for prediction of underlying phenotypes based on sequence data. Specifically, GoF approaches that generate information about mutations that do and do not give rise to phenotypic changes of interest provide critical training data for models, and GoF approaches are needed to validate model predictions in the context of the full virus. Importantly, other types of biophysical data generated through alternative experimental approaches are also critical for improving the accuracy of existing models. In addition to scientific advancements, full realization of the benefits derived from the use of computational models will require expanding the sequencing capabilities of influenza surveillance networks as described above.

The utility and limitations of different approaches for evaluating the transmissibility and virulence of circulating animal influenza viruses are summarized in Table 15.18 below. Both the direct measurement of virus phenotypes in the laboratory and the prediction of underlying phenotypes from genotype, either through sequence inspection for molecular markers or computational modeling approaches, have inherent strengths and limitations. Namely, the generation of phenotypic data will always be delayed by the need to ship clinical samples or viral isolates, and viruses may acquire adaptive changes that alter their phenotypic properties during isolation. However, direct measurements of phenotypic properties are invaluable. In contrast, as sequence data is increasingly available from NICs and other "base" level diagnostic laboratories, the application of predictive methods will enable the rapid generation of phenotypic "data" that reflects the properties of viruses present in clinical samples, allowing for more rapid characterization of emerging influenza viruses. However, due to the inherent uncertainties associated with predictions, the subsequent confirmation of predictions through phenotypic testing is critical. Therefore, virological data and sequence-based predictive data are complementary, and

consideration of both will strengthen the timeliness and accuracy of assessments of virus properties that contribute to pandemic risk.

Table 15.18. Summary of the Benefits of GoF Approaches That Enhance Transmission and Virulence in Mammals		
Surveillance benefits – Aid Evaluation of the Transmissibility and Virulence of Circulating Animal Influenza Viruses		
Approach	**Benefits**	**Limitations**
GoF #1: Support the development of rapid assays for phenotypes underlying mammalian adaptation, transmissibility, and virulence	• Provides a direct readout of phenotypes underlying mammalian adaptation, transmissibility, and virulence • Could expand the quantity of phenotypic characterization data available: o High-throughput assays will enable the characterization of a large number of surveillance isolates • Could increase the timeliness of phenotypic characterization data available: o Relatively simple execution of rapid phenotypic assays relative to ferret testing experiments will enable testing at NICs, abrogating the need to ship samples to WHOCCs for characterization • Enables detection of viruses that are "partially adapted" o Viruses that exhibit changes in one or more underlying phenotypes	• Reliable rapid phenotypic assays do not yet exist, and their future validity depends on scientific advancements o Timeframe for establishing that knowledge is uncertain, likely to be long-term • Broad utility of rapid phenotypic assays will depend on whether mechanisms underlying mammalian adaptation, transmissibility, and virulence are conserved across different strains o Not yet well-understood • The need to conduct assays involving whole virus under BSL-3 conditions will limit the number of diagnostic labs that can carry out these assays

Table 15.18. Summary of the Benefits of GoF Approaches That Enhance Transmission and Virulence in Mammals		
Surveillance benefits – Aid Evaluation of the Transmissibility and Virulence of Circulating Animal Influenza Viruses		
Approach	**Benefits**	**Limitations**
GoF #2: Strengthen the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence	• Could increase the accuracy of phenotypic characterization data: o Clinical samples can be directly sequenced • Could increase the timeliness of phenotypic characterization data: o NICs and other field diagnostic labs are increasingly capable of sequencing virus samples, abrogating the need to ship samples to WHOCCs for characterization • Could expand the quantity of phenotypic characterization data: o As sequencing becomes cheaper and easier, whole genome sequencing of viruses collected through surveillance will become increasingly common • Enables detection of viruses that are "partially adapted" o Viruses that exhibit changes in one or more underlying phenotypes • Molecular marker data are currently used to interpret surveillance data o New data can be incorporated into the process in the immediate term	• Significant scientific uncertainties compromise the current utility of molecular markers for mammalian adaptation, transmissibility, and virulence o Time frame for establishing that knowledge is uncertain, likely to be long-term • Use of molecular markers is inherently predictive • Full realization of benefits depends on expanding sequencing capabilities at NICs, as well as increasing the number of viruses that are subjected to whole genome sequencing and the number of clinical samples that are directly sequenced

Table 15.18. Summary of the Benefits of GoF Approaches That Enhance Transmission and Virulence in Mammals

Surveillance benefits – Aid Evaluation of the Transmissibility and Virulence of Circulating Animal Influenza Viruses		
Approach	**Benefits**	**Limitations**
GoF #3: Support development of computational models for predicting phenotypes underlying mammalian adaptation, transmissibility, and virulence based on sequence	• Increase the accuracy of phenotypic characterization data: o Clinical samples can be directly sequenced • Increase the timeliness of phenotypic characterization data: o NICs and other field diagnostic labs are increasingly capable of sequencing virus samples, abrogating the need to ship samples to WHOCCs for characterization • Increase the quantity of phenotypic characterization data: o As sequencing becomes cheaper and easier over time, whole genome sequencing of viruses collected through surveillance will become increasingly common • Enables detection of viruses that are "partially adapted" o Viruses that exhibit changes in one or more underlying phenotypes	• Reliable computational models for phenotypes underlying mammalian adaptation, transmissibility, and virulence do not yet exist, and their future validity depends on scientific advancements o Timeframe for establishing that knowledge is uncertain, likely to be long-term • Use of computational models is inherently predictive • Full realization of benefits depends on expanding sequencing capabilities at NICs, as well as increasing the number of viruses that are subjected to whole genome sequencing and the number of clinical samples that are directly sequenced
Alt-GoF #1: Phenotypic evaluation of mammalian adaptation, transmissibility, and virulence in ferrets or other appropriate animal models	• Provides direct readout of infectivity, transmissibility, and virulence in appropriate animal models	• Assays are unable to detect when viruses have acquired underlying phenotypic changes that are necessary but not sufficient to alter infectivity, transmissibility, and virulence in mammals (i.e., "partially adapted" viruses) • The number of viruses that can be characterized is limited by the availability of surveillance isolates • Sample shipping delays due to political and regulatory factors delay the generation of phenotypic data o Due to the technical expertise and biocontainment conditions required for these assays, they are currently conducted at WHOCCs only

15.3.5 Benefits to Decision-Making in Public Health Policy

GoF approaches that enhance the infectivity and transmissibility of animal influenza viruses in representative animal models have potential to benefit pandemic preparedness planning in two ways. First, the demonstration that avian influenza viruses can evolve the capacity for more efficient transmission in mammals may, in and of itself, stimulate interest and investment in pandemic preparedness initiatives. The second benefit derives from GoF benefits to surveillance. Analysis of the phenotypic properties of animal influenza surveillance isolates plays a critical role in assessment of their pandemic risk, as described in detail below. In turn, pandemic risk assessments inform decision-making about how to invest in public health preparedness activities for influenza pandemics. Thus, GoF-derived improvements to the analysis of influenza surveillance data could have downstream benefits to decision-making in public health policy. This section evaluates the potential benefits of each type of GoF data, relative to alternative approaches, in turn.

15.3.5.1 Benefits of "Proof of Principle" GoF Research That Demonstrates the Capacity of a Virus to Evolve More Efficient Transmissibility in Representative Animal Models

Researchers have suggested that the "proof of principle" demonstration that an animal influenza virus can evolve the capacity for airborne transmission in a laboratory setting, as a blunt indicator of the pandemic potential of the virus, could inform government interest and investment in pandemic preparedness initiatives. However, pandemic preparedness activities at the US CDC and ASPR, including BARDA, did not change in the wake of the 2012 demonstration that H5N1 could evolve the ability to transmit via the airborne route between ferrets, suggesting that this is not a real benefit.[1458,1459,1460] CDC and BARDA representatives noted that the level of resources dedicated to H5N1 preparedness was already high at the time those papers were published, as many CVVs had been developed and a quantity of pre-pandemic vaccine doses had been developed stockpiled.[1461] Thus, there may have been minimal room for increasing the level of USG investment in preparedness for that virus sub-type. However, pandemic preparedness activities also did *not* change in response to the laboratory demonstration that avian influenza H9N2 could acquire the capacity for airborne transmission in ferrets, which provides an instructive comparison.[1462] At that time, multiple CVVs for H9N2 had been developed, but decision-makers had chosen not to proceed further along the pre-pandemic vaccine production pipeline because H9N2 had caused fewer and milder cases than H5N1.[1463] This finding indicates that the epidemiological differences between H5N1 and H9N2 human infections were responsible for the initial differences in the level of resources dedicated to preparedness for each virus. However, the laboratory transmission results did *not* change this decision, suggesting that for viruses that have already caused human infections, additional laboratory data will not significantly influence decision-making related to pandemic preparedness.[1464] USG representatives involved in pandemic preparedness indicated that the response to the demonstration that an animal virus that has not yet caused human infections can evolve the capacity for airborne transmission would also be minimal, due to the lack of certainty about whether laboratory results translate to humans in nature.[1465] If the virus were known or suspected to be circulating in animal populations in the US, enhanced

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1458 Imai M *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486: 420-428

1459 Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

1460 (2015i) Interviews with CDC, ASPR, and BARDA representatives.

1461 (2015j) Interviews with CDC and BARDA representatives.

1462 Sorrell EM *et al* (2009) Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus. *Proc Natl Acad Sci U S A* 106: 7565-7570

1463 (2015j) Interviews with CDC and BARDA representatives.

1464 Ibid.

1465 Ibid.

surveillance might be undertaken to better understand the prevalence and geographic distribution of the virus in nature. However, the result would be highly unlikely to trigger investments in pre-pandemic vaccine development. Notably, this result may impact pandemic preparedness planning in developing countries in which high-risk viruses are circulating, as discussed in the "Globalization of Benefits" Section 15.9.

15.3.5.2 Benefits of GoF Research That Informs Pandemic Risk Assessments

The second mechanism through which GoF approaches can benefit pandemic preparedness planning is through pandemic risk assessments, downstream of GoF benefits to surveillance. As discussed in section 15.3.4, GoF approaches have potential to benefit virological surveillance (i.e., by supporting the development of rapid phenotypic assays) as well as genetic surveillance (i.e., by strengthening the predictive value of molecular markers for phenotypic properties of concern and by improving computational models for predicting phenotype from genotype). The use of molecular markers for phenotypic properties of concern is currently incorporated into the risk assessment process, as described in detail below. As neither rapid assays nor robust computational models for relevant phenotypes exist, how results from notional future assays/models would be considered in risk assessments is uncertain. Thus, the potential benefits of rapid phenotypic assays or computational models to pandemic risk assessments is not formally evaluated in this section, but a discussion of how results from either could contribute to the risk assessment process is provided at the end of the section.

This section analyzes the value of using molecular marker data relative to other types of data that are considered in the pandemic risk assessment process (i.e., epidemiological and ecological data), which provides an "upper bound" to the public health benefits that can be achieved through GoF improvements to surveillance. First, to provide context for this analysis, current strategies for pandemic risk assessments are reviewed, and shortcomings in existing processes are highlighted.

15.3.5.2.1 Background – Pandemic Risk Assessment and Strategies for Decision-Making About Investments in Pandemic Preparedness

Influenza pandemics occur when a novel influenza virus becomes transmissible in human populations with limited or no pre-existing immunity. Due to the complex interplay between virus, host, and ecological factors that shape viral evolution in nature, predicting the timing of the next influenza pandemic and the strain that causes it is not possible.[1466] Nonetheless, given the high public health burden associated with annual influenza epidemics and past influenza pandemics (see chapter 5), the US government undertakes influenza pandemic preparedness activities to bolster US capabilities for rapid detection of novel influenza events and to limit the spread of disease, death, and potential societal impacts if/when the next influenza pandemic occurs.[1467] Some preparedness efforts target particular influenza strains or sub-types, including the development of novel diagnostics, enhanced animal or public health surveillance, and the development of pre-pandemic vaccines, while others are largely strain-agnostic, such as stockpiling antivirals. In particular, the development of pre-pandemic vaccines is a key aspect of pandemic preparedness because influenza vaccination is the primary public health strategy for reducing influenza-associated morbidity and mortality during outbreaks.[1468]

[1466] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

[1467] Ibid.

[1468] Ampofo WK *et al* (2013b) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

As resources for pandemic preparedness efforts are limited, a major challenge is determining how resources for strain-specific investments should be allocated, in particular for the development of pre-pandemic vaccines. The US National Strategy for Pandemic Influenza (2005) calls for a risk- and evidence-based approach to guide comprehensive planning and response efforts.[1469] To that end, the CDC, in collaboration with subject matter experts in influenza virology, diagnosis, epidemiology, ecology, and laboratory research in animal and human influenza, developed a framework for assessing the relative risk posed by emerging influenza viruses and an accompanying tool – the Influenza Risk Assessment Tool (IRAT). Those results then inform prioritization of resources for preparedness efforts directed at particular strains/sub-types.

The IRAT provides a formal method for evaluating the relative risk posed by different emerging influenza strains (e.g., H5N1 versus H7N9).[1470,1471] This method is based on subject matter expert input about risk elements that govern the likelihood that a particular strain will adapt to efficiently transmit in human populations and the expected public health consequences of that emergence event. These risk elements can be broadly grouped into four categories:

- Elements relating to the properties of the virus (e.g., transmissibility and virulence),
- Elements relating to the attributes of host populations (e.g., the degree of pre-existing immunity),
- Elements relating to epidemiology, and
- Elements relating to ecological factors (e.g., the extent of human infections and the prevalence and geographic distribution of the virus in animal populations).

Selected elements will be described in more detail below. Risk elements pertaining to the properties of the virus are informed by virological data (e.g., transmission studies in ferrets) and by genomic data, including molecular marker data (e.g., whether molecular markers associated with enhanced transmissibility in ferrets are present in the viral genetic sequence). Individual risk elements have been weighted, based on SME input about their relative contribution to the likelihood and expected consequences of emergence of particular strains, and all elements are considered collectively to determine an overall risk score. Notably, the relative weighting factors for distinct risk elements are different for the "likelihood of emergence" and "consequences" parts of the tool.

Only some emerging viruses are subjected to formal risk assessments using the IRAT, and not all pandemic preparedness decisions related to those viruses are based on formal risk assessment scores. However, the risk elements outlined in the IRAT are considered when informally evaluating risks posed by emerging influenza viruses. Thus, the following analysis of how GoF benefits to surveillance could improve the pandemic risk assessment process and downstream decision-making represents the value of GoF insights to decision-making about preparedness for emerging influenza outbreaks in general.

15.3.5.2.2 Potential Benefits of GoF to Pandemic Risk Assessments: Utility and Limitations of Using Molecular Marker Data

GoF approaches have potential to improve the accuracy, timeliness, and quantity of phenotypic information generated by inspecting sequences for the presence of molecular markers for mammalian adaptation, transmissibility, and virulence. This section focuses on the utility and limitations of molecular marker data to the pandemic risk assessment process, relative to other types of data that are considered (e.g., virological data, epidemiological data, and ecological data).

1469 Homeland Security Council. (2005) National Strategy for Influenza. Washington, D.C.

1470 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

1471 Trock SC *et al* (2012) Development of an influenza virologic risk assessment tool. *Avian diseases* 56: 1058-1061

Molecular Marker Data

The genomic variation risk element includes consideration of the genetic diversity of animal influenza viruses, which includes the presence of known molecular markers for phenotypic properties of concern.[1472] Markers for phenotypes underlying mammalian adaptation, transmissibility, and virulence are considered most heavily,[1473] in conjunction with structural modeling to account for differences in genetic context, if appropriate. As described above, these analyses complement results from laboratory-based phenotypic assays, particularly in cases when clinical isolates can be directly sequenced. The major strength of this analysis is that sequence data are now the fastest, most reliable data produced at NICs and other field laboratories where animal influenza viruses of concern are circulating. For example, the Chinese government uploaded the sequences of the viral isolates from the first three human cases of H7N9 influenza promptly, before additional information about the phenotypic properties of the virus was available. The US CDC received the wild type virus from China 12 days later, after which additional phenotypic testing could begin, resulting in a lag time for production of phenotypic data of several weeks relative to genetic data.[1474,1475] However, the predictive value of molecular markers is compromised by significant sources of scientific uncertainty associated with the functional generalizability of the markers and the linkage between underlying phenotypes and adaptation/transmissibility/virulence, as described above. Because of these uncertainties, molecular marker data contributes moderately to the risk assessment, relative to other factors. For example, in the three-virus relative risk assessment referenced above, findings related to epidemiology risk elements were about six-fold more important than findings in the genomic variation risk element. GoF approaches have the potential to improve the predictive value of molecular markers, but whether that will translate to an increased weight relative to other factors considered in the risk assessment is unknown.

15.3.5.2.3 Potential Benefits and Limitations of Alternative Pandemic Risk Assessment Factors

Virologic Data

The relative strengths and weaknesses of using molecular markers versus virological approaches to characterize the phenotypic properties of surveillance viruses were discussed extensively in Section 15.3.4. This section evaluates the utility and limitations of virologic data in the context of the overall pandemic risk assessment.

Several risk elements rely on laboratory data: receptor binding (preference for "human-like" α2,6 sialylated receptors, "avian-like" α2,3 sialylated receptors, or dual specificity), transmission in animal models, antiviral resistance, disease severity in animal models, and antigenic relationship between virus and existing CVVs/vaccines.[1476,1477] Although epidemiologic measurements also provide information about the severity and transmissibility of a virus, these phenotypes are difficult to measure accurately in nature, especially when a virus first emerges in human populations and epidemiological data are scarce. As performing human transmission and virulence studies using novel influenza viruses would be unethical, laboratory-generated phenotypic data critically complement epidemiologic observations. Accordingly, in a recent assessment of three influenza viruses (an avian H1N1 virus, a human isolate of H7N9, and a human isolate of H3N2v), these elements were highly weighted. For evaluating the

[1472] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

[1473] (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.

[1474] (2015p) Interview with CDC Representative.

[1475] Dormitzer PR. (2014) Synthetic Influenza Vaccine Viruses. *Session 5*. National Academy of Sciences Symposium on Potential Risks and Benefits of Gain of Function Research

[1476] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

[1477] Trock SC *et al* (2012) Development of an influenza virologic risk assessment tool. *Avian diseases* 56: 1058-1061

likelihood of emergence, transmission data were approximately two-thirds the value of data about the extent of human infections (the highest-value element), and receptor binding data were half the value of the human infection data. For evaluating potential consequences of emergence, disease severity was the most important risk element. (The disease severity risk score reflects the severity of human infections and the severity in appropriate animal models.)[1478] The major limitations associated with reliance on laboratory-generated phenotypic data were described above. In sum, the virus composition and/or sequence may change during the isolation process, such that assay results do not accurately reflect the characteristics of the viral species present in the original clinical sample, and political, logistical, and regulatory factors delay receipt of clinical specimens/viral isolates in US labs.

Epidemiologic Data

Three risk elements rely on epidemiologic data: human infections, disease severity (which is also informed by laboratory testing in animals), and population immunity (detection of pre-existing cross-reactive serum antibodies). The human infections and disease severity elements are the most important elements of the likelihood and consequences components of the IRAT, respectively, because the data directly reflect the properties of the virus in humans. However, there are several challenges associated with the interpretation of epidemiological data for pandemic risk assessments. When a novel virus first emerges, extrapolating virus properties from a limited number of human cases may be difficult. In particular, disease severity is often initially over-estimated because only severe cases interact with the public health system, and serological studies to ascertain population exposure are difficult and time-consuming to carry out.

Ecological/Environmental Factors

Finally, two risk elements involve ecological factors, which collectively consider the global distribution of the virus in animals: the number of species that can be and are infected and the potential extent of exposure between humans and those animal species. Other environmental information, such as the strength of the public health systems and the strength of the relationship between the public health and veterinary services sectors in countries in which the virus is circulating in animal populations, may also be considered. These elements are moderately important in the likelihood component and minimally contribute to the consequence component of the IRAT. Importantly, these elements reflect completely different aspects of risk than the elements based on phenotypic, genetic, and epidemiologic data.

15.3.5.2.4 Summary – Benefits of GoF Approaches to Pandemic Risk Assessments

GoF approaches have potential to benefit pandemic risk assessments by strengthening the predictive value of molecular markers for mammalian adaptation, transmissibility, and virulence, which are a component of the "genomic variation" risk element considered in the assessment. The relative importance of this element relative to other risk elements places a qualitative "upper bound" on the potential benefits of GoF research to pandemic risk assessments. Notably, because molecular marker data are currently incorporated into pandemic risk assessments, the benefits of GoF-derived improvements to the reliability of molecular marker data could be immediate.

The strengths and weaknesses of different types of data considered in a pandemic risk assessment are summarized in Table 15.19, below. Epidemiological data (alt-GoF) represent the most important input to the risk assessment, for both the likelihood and consequences of emergence component of the IRAT. Laboratory data about transmissibility and virulence in appropriate animal models and receptor binding

[1478] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

specificity also significantly contribute to the overall pandemic risk score. Genomic variation, which includes consideration of molecular marker data for mammalian adaptation, transmissibility, and virulence, is relatively less important. Given the caveats associated with epidemiological and virological data, subject matter experts involved in the pandemic risk assessment process emphasized the value of corroborating information about infectivity, transmissibility, and disease severity in humans or appropriate animal models with molecular marker data.[1479] Those genetic data can increase confidence in an estimate of risk adds certainty to decision-making downstream of the risk assessment, which is valuable.

Molecular marker data play a more important role in the risk assessment when a novel influenza virus first emerges in the human population. In this scenario, epidemiological data will be scant and sequence data are likely to be available before phenotypic data, as happened when avian influenza H7N9 emerged in China in March 2013. As a result, the use of molecular marker data enables a rapid risk assessment of the emerging virus, so that downstream response actions can be initiated more quickly if deemed appropriate. For example, a rapid risk assessment of H7N9 triggered the decision to immediately develop a candidate vaccine virus. Of note, this risk assessment was also influenced by epidemiological observations – namely, that multiple cases were reported in a short period of time, which hints at an outbreak and possible detection issues. This rapid assessment resulted in initiation of vaccine production three to four weeks earlier than if decision-makers had waited until complete phenotypic data were available. Specifically, the wild type H7N9 virus arrived at the US CDC from China 12 days after the sequences were published online, and characterizing the transmissibility and virulence of the virus in ferrets requires an additional one to two weeks. (Of note, experts "re-ran" H7N9 through the IRAT once phenotypic data had been generated, and the final score was relatively close to the initial score.) In the event of a pandemic, such a three to four week head start on vaccine production could significantly reduce pandemic-associated morbidity and mortality. For example, researchers estimate that deployment of vaccine two weeks earlier during the 2009 H1N1 pandemic would have prevented an additional ~600,000 cases (an approximately 60% increase in the number of cases prevented), while deployment of the vaccine four weeks earlier would have prevented an additional 1.4 million cases (an approximately 135% increase in the number of cases prevented).[1480]

International surveillance for influenza is improving, especially in the wake of the 2009 pandemic, but gaps remain, particularly in certain regions of the world (e.g., parts of Africa, regions experiencing political instability, etc.). The limited breadth of available surveillance data constrains the potential benefits of using pandemic risk assessments to guide decision-making about pandemic preparedness investments. That is, experts can only evaluate and prepare for pandemics caused by strains they know about. Mild disease cases, cases in remote areas, or cases in regions without strong surveillance and disease reporting systems are likely to be missed by existing passive surveillance systems for novel influenza cases. The viruses that cause these "hidden" cases could pose risks to human populations, in which case the public would benefit from pandemic preparedness initiatives targeting those viruses. Additionally, an improved ability to detect mild cases caused by known high-risk viruses, such as H5N1, would increase the accuracy of risk assessments for these viruses by strengthening the quality of the underlying epidemiological data. For these reasons, all stakeholders interviewed for this report, including influenza researchers, public health personnel, and USG public health policy representatives, agreed that there is a clear need to strengthen and expand influenza surveillance networks. Importantly, expanded surveillance alone is not sufficient to improve pandemic risk assessments without concomitant improvements to the tools used for pandemic risk assessments, including the use of molecular marker

[1479] (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.
[1480] Borse RH *et al* (2013) Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. *Emerging infectious diseases* 19: 439-448

data. Thus, strong surveillance networks function as a co-factor that is needed for the full realization of GoF benefits to pandemic risk assessments.

As discussed in Section 15.3.4, GoF approaches can also benefit surveillance for animal influenza viruses by enabling the development of rapid assays for phenotypes underlying mammalian adaptation, transmissibility, and virulence, as well as by improving computational models for sequence-based predictions of underlying phenotypes. Either type of data could be used to corroborate information about transmissibility and virulence gleaned through ferret experiments. Given the variability inherent in animal experiments, in part because ferrets used for testing in different locations are genetically diverse, data about underlying phenotypes could strengthen the robustness of this phenotypic information. If the linkage between an underlying phenotype and adaptation/transmissibility/virulence is sufficiently strong, the underlying phenotype could be used as an individual component of the risk assessment, akin to the current sialic acid receptor binding specificity element. Both rapid phenotypic assays and computational models could inform evaluation of this kind of risk element. The fact that weights for the sialic acid receptor binding specificity, transmissibility, and disease severity elements are intermediate to high suggests that validated rapid phenotypic assays could add significant value to the pandemic risk assessment. However, the timeline for realization of this benefit is likely to be long-term. The benefits arising from rapid phenotypic assays depends on the discovery and validation of suitable underlying phenotypes and the development and validation of an appropriate rapid phenotypic assay. The benefits arising from the use of computational models depend on the development of reliable models, which will likely prove to be a significant scientific challenge. The timescales for these scientific and technical innovations are unknown.

Table 15.19. Summary of the Benefits of GoF Approaches That Enhance Transmission and Virulence in Mammals

Benefits to Decision-Making in Public Health Policy – Inform Pandemic Risk Assessments of Circulating Animal Influenza Viruses

Approach	Benefits	Limitations
GoF #1: Genomic variation risk element: • Information about molecular markers for mammalian adaptation, transmissibility, and virulence • Information about reassortment	• Corroborate laboratory data about mammalian adaptation, transmissibility, and virulence o Increases **certainty** in decision-making downstream of the pandemic risk assessment • Enables **rapid** risk assessment of newly emerged viruses o Sequence data are typically the fastest and most reliable data available from diagnostic laboratories where animal influenza of concern are circulating o Provides a head start on pre-pandemic vaccine development and other pandemic preparedness activities	• Predictive value of molecular markers is currently limited due to several sources of scientific uncertainty o **Moderate** contribution to overall risk score (e.g., five- to six- fold less important than epidemiology data)
Alt-GoF #1: Virological data: • Information about transmissibility and disease severity in ferrets • Information about sialic acid receptor binding specificity	• Provides a **direct** readout of infectivity, transmissibility, and virulence in appropriate animal models o Critical complement to epidemiological observations • **High** contribution to overall risk score o About two-thirds as important as epidemiology data, the most important element	• Results in animal models may not translate to human disease • Logistical, political, and regulatory factors delay sample shipment to WHOCCs and subsequent generation of phenotypic data o Data may not be available until *after* sequence data
Alt-GoF #2: Epidemiology data: • Information about the number and severity of human infections • Information about the degree of pre-existing immunity in human populations	• Data directly reflects the properties of the virus in humans • **Highest** contribution to overall risk score, out of all risk elements considered • Information about pre-existing immunity in the population complements information about properties of the virus and ecological factors	• Reliable measurement of epidemiological factors when new viruses first emerge in human populations is difficult o Early data may be incomplete and/or inaccurate

Table 15.19. Summary of the Benefits of GoF Approaches That Enhance Transmission and Virulence in Mammals		
Benefits to Decision-Making in Public Health Policy – Inform Pandemic Risk Assessments of Circulating Animal Influenza Viruses		
Approach	**Benefits**	**Limitations**
Alt-GoF #3: Ecological data: • Information about the global distribution of the virus in animal populations and the nature of human exposure to infected animals	• Information about ecological factors complements information about properties of the virus and of the host population • **Moderate** contribution to likelihood that a virus will emerge in human populations	• Minimal contribution to potential consequences of virus emergence in human populations • Gaps in surveillance in animal populations compromise accuracy of information

15.3.5.2.5 Public Health Impacts of Pandemic Risk Assessments

Formal pandemic risk assessments are carried out to help prioritize resources for investments in pre-pandemic vaccine development. Informal risk assessments may also guide investments in other pandemic preparedness initiatives, such as sending a team of CDC experts abroad to investigate a concerning cluster of zoonotic influenza infections in humans.

Strain-specific diagnostics are not developed in response to pandemic risk assessments (formal or informal). The process for developing influenza diagnostics is well-established, and developing new diagnostics is rapid and requires minimal resources relative to investments in pre-pandemic vaccine development.[1481,1482] A single human infection with a novel influenza sub-type is sufficient to trigger the CDC to design primers and probes for a new diagnostic assay, and epidemiological data (i.e., the number and severity of infections) also govern whether the CDC will undertake validation and subsequent FDA licensing of the new assay.[1483] Pandemic risk assessments do not trigger enhanced influenza surveillance in the US either. The US public health system already has a surveillance system in place for detection of novel influenza A infections, which must be reported to the CDC within 24 hours.[1484]

GoF approaches contribute to decision-making about pandemic preparedness activities insofar as molecular marker data informs pandemic risk assessments. Thus, the value of GoF-derived data relative to alternative factors that contribute to the risk assessment is the same as described for pandemic risk assessments, above. Independently of a pandemic risk assessment, GoF approaches also contribute to the selection of viruses used as the basis of pre-pandemic vaccines. Notably, completely different strategies may also achieve the same ultimate public health goals as pre-pandemic vaccine development and testing antiviral efficacy against high-risk strains. Below, the contribution of GoF approaches to decision-making related to pre-pandemic vaccine development and testing antiviral efficacy against high-risk strains is evaluated, as well as alternative approaches that aim to achieve the same public health goals.

Pre- Pandemic Vaccine Development

Because existing influenza vaccines are strain-specific, pre-pandemic vaccines are developed to target particular groups of high-risk strains. Depending on the overall level of risk associated with a particular virus, the US government will fund development of a pre-pandemic vaccine through various stages of the vaccine production pipeline. Each of the following steps requires an escalating expenditure of resources: CVV development, conduct of pre-clinical vaccine studies in animals, manufacture of clinical trial lots of vaccine, conduct of human clinical trials, stockpiling of vaccine, and priming the population against the novel influenza virus (e.g., administering vaccine in advance of a pandemic).[1485] Collectively, these investments will increase the availability of vaccines during a pandemic. Developing pre-pandemic CVVs could save up to nine weeks (the time needed to develop and test a CVV), developing a vaccine seed strain could shave off another two to three weeks, and carrying out pre-clinical studies in animals or

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[1481] Diagnostic assays for animal influenza viruses are real-time PCR-based. Diagnostic targets include the M gene (a generic marker for influenza A viruses) and the HA gene (for sub-typing), and may also include the NA gene. The development of a new diagnostic assay simply requires designing primers and probes for these genes.

[1482] 2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

[1483] Ibid.

[1484] Council of State and Territorial Epidemiologists. CSTE List of Nationally Notifiable Conditions. https://c.ymcdn.com/sites/cste.site-ym.com/resource/resmgr/CSTENotifiableConditionListA.pdf. Last Update August 2013. Accessed November 6, 2015.

[1485] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

human clinical trials could shorten production timelines by as much as 12 to 14 weeks.[1486] Farther down the vaccine production pipeline, stockpiling bulk antigen would allow for near-immediate deployment of vaccine following emergence of a pandemic strain, while priming the population provides advanced protection. In addition, manufacturers' experience with production of the vaccine would likely streamline subsequent large-scale production during the pandemic.[1487] Although the pre-pandemic vaccine strain is unlikely to exactly match the strain that emerges to cause a pandemic, use of adjuvants and prime-boost regimens broaden the protection that can be achieved using a strain-specific vaccine, such that pre-pandemic vaccines are highly likely to provide some level of protection against infection with a similar strain.[1488,1489,1490,1491,1492] Notably, resources limit the scope of the USG's investment in pre-pandemic vaccines, highlighting the need for strategies to prioritize vaccine development for the many influenza viruses circulating in nature that have spilled over into human populations.[1493]

As described above, molecular marker data (derived from GoF approaches) may play an important role in the decision to develop a CVV for an animal influenza virus, though decisions about downstream stages of the vaccine production pipeline such as production of clinical lot material are likely to be delayed until virological data are available for consideration in the risk assessment.[1494] Once the decision is made to develop a CVV, multiple strains may be available to serve as the basis for the CVV. In the event that these strains have similar epidemiological and virological characteristics, the presence and type of molecular markers for mammalian adaptation, transmissibility, and virulence can serve to differentiate between strains. For example, the presence of markers associated with airborne transmissibility between ferrets supported the decision to develop a CVV for a particular H5N1 strain among several options, in response to an abrupt rise in the number of human cases in Cambodia in 2013.[1495,1496,1497] Thus, the application of molecular marker data enabled more granular decision-making than would have been possible based on other data sources alone, which is valuable because resource limitations constrain that number of CVVs that can be produced. This constraint is due to the fact that the number of facilities that can produce pre-pandemic CVVs using Good Manufacturing Processes (GMP) is limited and that CVVs used for vaccine production must undergo extensive safety and characterization testing, which is resource-intensive.[1498]

1486 (2015r) Rapid Medical Countermeasure Response to Infectious Diseases: Enabling Sustainable Capabilities Through Ongoing Public- and Private-Sector Partnerships: Workshop Summary. The National Academies Press.

1487 (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

1488 Ibid.

1489 (2015s) Influenza Vaccines. Interviews with Public Health Professionals Involved in Preventing and Responding to Influenza Outbreaks.

1490 Smith GE *et al* (2013) Development of influenza H7N9 virus like particle (VLP) vaccine: homologous A/Anhui/1/2013 (H7N9) protection and heterologous A/chicken/Jalisco/CPA1/2012 (H7N3) cross-protection in vaccinated mice challenged with H7N9 virus. *Vaccine* 31: 4305-4313

1491 Middleton D *et al* (2009) Evaluation of vaccines for H5N1 influenza virus in ferrets reveals the potential for protective single-shot immunization. *Journal of virology* 83: 7770-7778

1492 Khurana S *et al* (2010) Vaccines with MF59 adjuvant expand the antibody repertoire to target protective sites of pandemic avian H5N1 influenza virus. *Sci Transl Med* 2: 15ra15

1493 Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

1494 (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.

1495 Schultz-Cherry S *et al* (2014) Influenza Gain-of-Function Experiments: Their Role in Vaccine Virus Recommendation and Pandemic Preparedness. *MBio* 5

1496 Davis CT *et al* (2014) Use of highly pathogenic avian influenza A(H5N1) gain-of-function studies for molecular-based surveillance and pandemic preparedness. *MBio* 5

1497 Rith S *et al* (2014) Identification of molecular markers associated with alteration of receptor-binding specificity in a novel genotype of highly pathogenic avian influenza A(H5N1) viruses detected in Cambodia in 2013. *Journal of virology* 88: 13897-13909

1498 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

Notably, surveillance efforts for animal influenza viruses include cases of human disease and, to a lesser extent, surveillance of agricultural animal and wildlife populations. Both animal influenza viruses isolated from human infections as well as animal influenza viruses that have not yet caused human infections can be subjected to a risk assessment (formally or informally). However, because of the expense involved in each step of pre-pandemic vaccine production, none of the above steps are likely to be undertaken unless multiple human infections have occurred.[1499] As a result, although GoF approaches may aid the interpretation of surveillance data from animals, this proximal benefit will not lead to downstream investments in pre-pandemic vaccine development but rather is limited to deepening understanding of the risk associated with particular viruses. Utilizing animals as sentinels for human infections will require substantial expansion of animal influenza surveillance networks, as well as an increased understanding of how influenza viruses evolve in agricultural animal populations (in particular, the role of animal vaccination) and factors that govern evolutionary dynamics at the animal-human interface.

Several completely different strategies can increase the availability of vaccines during a pandemic, thus achieving the same ultimate public health goal. These strategies are described in detail in Section 15.2.4.3.3 and are briefly summarized here. First, a universal or broad-spectrum flu vaccine could be deployed in advance of a pandemic or could be rapidly deployed following the emergence of a novel pandemic strain. However, influenza and vaccinology experts disagree about the scientific feasibility of developing a universal vaccine, and one expert felt that a ten to twenty year time frame for development is optimistic. Second, several scientific and technical advancements could shorten production timelines for strain-specific vaccines, which would lead to faster vaccine availability during a pandemic. New vaccine platforms, such as recombinant vaccines, can be rapidly scaled up and have shorter production timelines than egg- and cell-based vaccines. However, the one recombinant vaccine on the market accounts for less than 1% of total seasonal influenza vaccine produced annually, and although several other virus-free vaccine platforms are in development, the length and expense of licensure processes for new vaccines will delay their widespread availability. Incorporating adjuvants into existing egg- and cell-based vaccines would allow for a smaller quantity of antigen to be used per vaccine dose, thus enabling production of the same number of doses in a shorter period of time. However, only one US-licensed pandemic vaccine includes adjuvants. Although an active area of research, adjuvanted vaccines must undergo standard FDA licensing procedures for new vaccines and thus are unlikely to be broadly available in the near future. Finally, GoF research that enhances virus production enables the development of higher-yield CVVs, which shortens vaccine production timelines by increasing the rate of bulk antigen production. Although this research can be immediately applied to improve vaccine production, this strategy provides the greatest benefit to the production of vaccines using poor-growing CVVs. However, as any strain may unexpectedly generate a low-yield CVV, such as the 2009 H1N1 pandemic strain, this benefit could significantly alleviate morbidity and mortality in the event that future pandemic strains are also grow poorly.

Field Investigations of Clusters of Zoonotic Influenza Infections Abroad

The CDC participates in missions to investigate zoonotic influenza cases or clusters of concern abroad, in conjunction with the WHO, OIE, Food and Agricultural Organization of the United Nations (FAO), and local Ministries of Health. The goal of these missions is to supplement foundational surveillance with in-depth investigations of ecological and environmental factors that may be contributing to spillover, including sources of human exposure to animal influenza viruses, whether and to what extent the virus is circulating in local animal populations, retrospective investigations of poultry deaths, and other factors. Collectively, these data improve understanding of the risk posed by the zoonotic influenza virus in that environment, which informs decision-making about other prevention and preparedness activities (such as

[1499] (2015c) Interview with USG representative involved in pandemic risk assessment and decision-making about investments pandemic preparedness initiatives.

whether to develop a pre-pandemic CVV). Recent examples include missions to Cambodia to investigate an abrupt rise in human H5N1 infections in 2013, to China in 2013 to investigate the initial wave of H7N9 human infections, and to Cairo, Egypt in March of 2015 to investigate the dramatic increase in the number of human cases of H5N1 infection recorded at the end of 2014 leading into the first few months of 2015.[1500,1501] The decision to send a CDC team abroad is informed by an assessment of whether the sequences of human isolates contain molecular markers for mammalian adaptation, virulence, and transmissibility. Similar to a formal risk assessment, this decision is driven by epidemiologic data but the presence of molecular markers of concern increases adds value by increasing certainty in decision-making. In addition, consideration of molecular marker data may stimulate increased attention to investigations of the local animal population and human interactions with infected animals, undertaken to better understand how ecological and environmental factors are influencing the evolution of the virus in that area.

15.4 Influenza Viruses: Detailed Analysis of the Benefits of GoF Research that Enhances Virulence

15.4.1 Overview of Influenza GoF Landscape

This assessment describes the benefits of GoF experimental approaches that are reasonably anticipated to enhance the morbidity or mortality of influenza viruses in appropriate animal models. In this section, we provide an overview of GoF approaches in this phenotypic category and describe the scientific outcomes and/or products of each approach.

15.4.1.1 Serial Passaging of Viruses in Cell Culture or Animal Models

Serial passaging of viruses in cell culture or animals selects for viruses with enhanced fitness or virulence, respectively. This approach is performed for three purposes. First, serial passaging is utilized to develop animal models for studying the mechanistic basis of flu-associated morbidity/mortality and for medical countermeasure development. Second, this approach enables the identification of mutations that are associated with enhanced fitness/virulence, which provides a foundation for follow-up studies that investigate the mechanistic basis of pathogenicity. These studies can also provide insight into host mechanisms underlying disease pathology by correlating host immune responses with morbidity and mortality measures. Third, the serial passaging approach is used to determine whether attenuated strains are capable of recovering virulence upon passage *in vitro* or *in vivo*. This third type of serial passaging study may be carried out using live attenuated influenza vaccine (LAIV) candidates, as an important aspect of safety testing prior to human clinical trials. In addition, these studies may be conducted using strains with fitness defects arising from the acquisition of antiviral resistance or other GoF phenotypes, in order to gain insight into the likelihood that these strains will persist and spread in nature. All types of serial passaging studies may be performed with seasonal or animal (i.e., avian and swine) viruses, and animals such as mice, ferrets, and swine may be used. Of note, serial passaging studies involving attenuated strains simply increase the human health risk of the attenuated strain to approach that of wild type strains.

15.4.1.2 Forward Genetic Screen to Identify Mutations That Enhance Fitness/Virulence

Forward genetic screens involve random mutagenesis of genetic regions predicted to contribute to fitness/virulence or comprehensive reassortment of parental gene segments from two viruses, followed by

[1500] (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.
[1501] Davis CT *et al* (2014) Use of highly pathogenic avian influenza A(H5N1) gain-of-function studies for molecular-based surveillance and pandemic preparedness. *MBio* 5

characterization of the fitness or virulence of mutants in appropriate mammalian model systems to select for mutant viruses with enhanced fitness/virulence. Sequencing emergent viruses enables the identification of mutations or gene segments that enhance the fitness/virulence of viruses, which provides a foundation for follow-up studies that investigate the mechanistic basis of pathogenicity in mammals. These studies are performed using human seasonal viruses, the 1918 H1N1 pandemic virus, and animal viruses. A variant of this approach involves the use of strains with impaired fitness due to the evolution of antiviral resistance, to determine whether strains can recover fitness through the acquisition of compensatory mutations, which has been performed using seasonal strains.

15.4.1.3 Targeted Modification of Viruses to Introduce Traits That Are Expected to Enhance Fitness/Virulence in Mammals

Targeted genetic modification of viruses, namely site-directed mutagenesis and/or reassortment, to introduce genetic traits that are expected to enhance the fitness/virulence of viruses followed by characterization of the fitness/virulence of mutants in cell culture or animal model systems, respectively, may lead to the generation of viruses with enhanced fitness/virulence in mammals. This approach is performed for two purposes: (1) to determine whether a previously characterized underlying genetic or phenotypic trait, such as evasion of a particular innate immune response, contributes to the complex phenotype of pathogenicity and (2) to confirm that a particular mutation or gene segment is necessary and sufficient to enhance the fitness/virulence of viruses in appropriate model systems. Traits that are associated with enhanced pathogenicity may be discovered through GoF approaches, such as serial passaging, or alt-GoF approaches, such as random mutagenesis followed by screening for attenuated virulence (Loss of Function). As above, this information provides a foundation for follow-up studies investigating the mechanistic basis of pathogenicity. These studies are performed using human seasonal viruses, the 1918 H1N1 pandemic virus, and animal viruses.

We note that the relationship between viral fitness and pathogenicity is complex and that many of the viral traits that contribute to fitness, either directly or indirectly, mediate pathogenicity. As a result, serial passaging of viruses in animals may select for both enhanced fitness and enhanced virulence. However, enhanced viral fitness *in vivo* does not necessarily translate to high pathogenicity, as seasonal influenza viruses do not display the morbidity and mortality displayed during infections with zoonotic influenza viruses such as H5N1, but grow to a high titer.

15.4.2 Overview of the Potential Benefits of GoF Experiments Involving Coronaviruses

Here we evaluate whether any of the GoF Influenza approaches have the potential to benefit each of the general benefit areas described in the NSABB's "Framework for Conducting Risk and Benefit Assessments of Gain of Function Research." We also describe additional benefit areas we identified during our research. Each potential benefit will be analyzed in detail below.

15.4.2.1 Scientific Knowledge

GoF approaches have the potential to benefit scientific knowledge in several ways. First, GoF approaches provide insight into the mechanistic basis of pathogenicity, including the identification of viral and host traits that contribute to pathogenicity. Second, information about compensatory mutations that rescue the growth of antiviral resistant strains provides a foundation for follow-up studies investigating the mechanistic basis of the enhanced growth phenotype, thereby benefiting scientific knowledge about the mechanisms underlying recovery of fitness in attenuated strains as well as the mechanistic interplay between different virus phenotypes. Finally, viruses with enhanced virulence developed using GoF approaches can be used as tools to understand how the host immune response contributes to morbidity

and mortality observed during influenza infections, representing an indirect benefit of GoF approaches to scientific knowledge.

15.4.2.2 Surveillance

GoF approaches that lead to the identification of molecular markers for enhanced pathogenicity have the potential to inform the interpretation of wildlife, agricultural animal, and public health surveillance information. Specifically, determining the presence (or absence) of particular mutations or of amino acid substitutions are particular sites is one aspect of evaluating the risk posed by circulating animal influenza viruses. Risk assessments based on evaluation of genetic surveillance data, as well as other types of data, then inform decision-making related to public health preparedness for novel influenza outbreaks, as discussed below.

GoF approaches that lead to the identification of compensatory mutations that rescue the fitness of antiviral-resistant strains with impaired growth do not benefit surveillance. Because of the high mutation rate of influenza viruses, influenza surveillance experts expect that antiviral resistant strains that initially exhibit impaired fitness can readily acquire compensatory mutations that rescue growth. Thus, experts simply track the presence of antiviral resistance markers, and the additional presence of absence of a known compensatory mutation does not increase or decrease the level of risk associated with the antiviral resistance marker.

15.4.2.3 Vaccines

GoF approaches have potential to benefit the development of vaccines in three ways:

- Serial passaging of candidate live attenuated vaccine strains in animals is used to test whether strains recover virulence upon growth *in vivo*, which is an important aspect of vaccine safety.
- GoF approaches enable the identification of conserved virulence determinants in the HA and NA proteins. These markers may be removed vaccine viruses through targeted deletion or mutagenesis, as is commonly done for the multi-basic cleavage site present in the HA proteins from some avian influenza strains, which may improve the efficacy and safety of the vaccine production process.
- Viruses with enhanced virulence, generated through GoF approaches, can be used as challenge viruses for vaccine efficacy studies, to facilitate the development of vaccines that can protect against severe disease.

15.4.2.4 Therapeutics

GoF approaches have potential to benefit the development of influenza therapeutics in two ways:

- GoF approaches that provide insight into viral and host traits that contribute to virulence identify potential targets for next-generation therapeutics (either targeting the virus or the host), and
- Viruses with enhanced virulence, generated through GoF approaches, can be used as challenge viruses for therapeutic efficacy studies, to facilitate the development of therapeutics that can ameliorate severe disease.

15.4.2.5 Diagnostics

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[1502]

15.4.2.6 Informing Policy Decisions

GoF approaches that lead to the identification of molecular markers for enhanced pathogenicity contribute to assessments of the pandemic risk posed by circulating animal influenza viruses, which are based on genetic surveillance data and several other types of data (e.g., epidemiologic data, phenotypic data, etc.). These assessments inform policy decisions related to public health preparedness for novel influenza outbreaks, including whether to develop and publicize messaging about risk factors for contracting animal influenza infections and practices for mitigation risks, whether to enhance surveillance of animals, and whether to develop pre-pandemic vaccines.

15.4.2.7 Economic Benefits

GoF benefits to the development of new vaccines and therapeutics could have downstream economic benefits. We did not explicitly evaluate economic benefits in this report.

15.4.3 Benefits of GoF to Scientific Knowledge

15.4.3.1 Scientific Knowledge Gap 1: What Are the Viral Genetic and Phenotypic Traits That Underlie Pathogenicity in Mammals? What Are the Host Factors That Contribute to Enhanced Pathogenicity as Well as Infection-Associated Morbidity and Mortality?

15.4.3.1.1 Introduction

The pathogenesis of influenza viruses reflects the complex interactions between viral and host factors and is the result of both the virus's ability to cause disease and the host's response to viral infection. From the virus perspective, pathogenicity is a complex phenotype defined by the combined effects of many underlying viral phenotypes including cell and tissue tropism, cytotoxicity, and replicative fitness. From the host perspective, the immune response is essential for inhibiting viral replication, as attenuated immune responses, such as those in immunocompromised hosts, fail to control infection. However, overly robust responses can result in severe immunopathology. The interplay between virus and host starts with the initiation of the early antiviral immune responses, leading to the recruitment of immune cells and the stimulation of adaptive immunity. Unsurprisingly, influenza viruses have several mechanisms to overcome this barrier, which contribute to fitness *and* pathogenicity and define the underlying phenotype of immune evasion. Of note, NS1 performs an array of tasks that inhibit detection by the host immune system and initiation of early immune responses, thereby providing opportunity for viral replication. Other viral proteins that contribute to pathogenicity by immune evasion and immune antagonism include PB1-F2, which induces host cell death and alters inflammatory responses. While advances in research have revealed functions of specific influenza proteins and genetic traits that contribute to virulence, the fact that overlapping and distinct mechanisms drive virulence in different strains and that a given genetic trait or protein may exhibit distinct functions in different genetic contexts complicate the translation of findings to other virus backgrounds. In particular, these differences pose challenges for comparing high

[1502] New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

and low pathogenicity strains. Accordingly, much remains to be elucidated on the interplay between virus-host interactions in defining pathogenesis.

In addition to immune evasion and antagonism, influenza viruses utilize a variety of other factors that result in enhanced virulence. The HA protein contributes to disease severity by initiating viral attachment and infection, and thus plays a large role in defining whether infections remain localized or become systemic, which greatly impacts pathogenicity and disease outcomes.[1503,1504,1505] Multiple aspects of HA function contribute to virulence. For example, the HA multibasic cleavage site, found in HPAI strains, influences tissue tropism by defining the sensitivity to tissue specific proteases that are required for its activation during infection. Other phenotypic traits that contribute to enhanced pathogenicity include polymerase activity and replicative fitness in mammalian cells due to adaptive mutations in the ribonucleoprotein complex (e.g., the PB2 E627K mutation) which enables replication at the lower temperatures observed in the human respiratory tract relative to the avian digestive tract. These examples emphasize the complexity of the relationship between viral fitness and pathogenicity as well as the fact that multiple, partially redundant mechanisms contribute to phenotypes underlying pathogenicity. Considerable gaps in knowledge remain about the molecular basis and role of each underlying phenotype in defining pathogenicity and associated disease outcomes, including systemic infection and severe immunopathology. In particular, the relationship between fitness and pathogenicity is poorly understood, as enhanced viral fitness *in vivo* does not necessarily translate to high pathogenicity. Further complicating this field of study is the fact that the viral genetic and phenotypic traits that contribute to enhanced virulence are not conserved in all high pathogenicity strains, suggesting that viruses may have distinct mechanisms of pathogenesis.

The cumulative effects of the interplay between virus and host shape the pathogenicity and severity of disease accompanying infection. Due to the complexity of the immune response and the diversity of immune responses observed in humans, attributable to variability in underlying genetic traits, previous exposures to influenza, and other environmental factors, there are considerable gaps in understanding how host factors ameliorate or potentiate morbidity and mortality associated with influenza virus infection. This is further complicated by a lack of knowledge about how early and late immune responses to primary infection shape tissue remodeling during viral clearance and resolution of the immune response.[1506] Another knowledge gap in this area is a lack of understanding about the mechanisms underlying patient susceptibility to and the outcomes of secondary bacterial infections, which significantly contribute to influenza-associated morbidity and mortality. In all cases, the identification and characterization of host factors that are necessary for viral and bacterial clearance independent of observed immunopathology is highly sought. By differentiating between these factors, uncoupling deleterious and protective effects of the immune response through host-targeted therapeutics may be possible.[1507]

The underlying genetic and phenotypic traits that enable efficient infection and drive pathogenicity are poorly understood, particularly because of the complex interplay among virus gene segments and between virus and host factors. Many host and viral factors synergize to exacerbate pathology, making mechanisms difficult to tease apart. Considerable gaps in knowledge remain about the molecular basis

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1503 Bottcher-Friebertshauser E *et al* (2014) The hemagglutinin: a determinant of pathogenicity. *Current topics in microbiology and immunology* 385: 3-34

1504 Kuiken T *et al* (2012) Pathogenesis of influenza virus infections: the good, the bad and the ugly. *Current opinion in virology* 2: 276-286

1505 Kash JC, Taubenberger JK (2015) The role of viral, host, and secondary bacterial factors in influenza pathogenesis. *The American journal of pathology* 185: 1528-1536

1506 Damjanovic D *et al* (2012) Immunopathology in influenza virus infection: uncoupling the friend from foe. *Clinical immunology (Orlando, Fla)* 144: 57-69

1507 Ibid.

and the role of each underlying phenotype in the context of the host response and viral fitness. Moreover, there is limited understanding of the host factors that contribute to protective versus deleterious outcomes. Insight into virus-host interactions is needed to advance in-depth understanding of virulence and pathogenesis of influenza viruses.

15.4.3.1.2 Potential Benefits and Limitations of GoF Approaches

Several GoF approaches can be used to discover the genetic and phenotypic markers underlying enhanced pathogenicity of influenza viruses:

- Targeted genetic modification to introduce novel genetic changes that are expected to contribute to pathogenicity by either site-directed mutagenesis or targeted reassortment (often between animal-origin or human pandemic and human seasonal strains),
- Forward genetic screens involving random mutagenesis or comprehensive reassortment followed by selection for enhanced virulence, or underlying phenotypes, and
- Serial passaging in appropriate animal models or mammalian cells to select for viruses with enhanced pathogenicity.

Collectively, these approaches enable the identification of genetic changes that are sufficient to confer enhanced pathogenicity in representative model systems. The GoF approaches described here also provide insight into host response pathways that contribute to underlying disease pathology. These approaches can be carried out in cell culture or in animal model systems, but the former is limited to the investigation of phenotypes underlying pathogenicity, such as replicative fitness and cell-specific immune evasion pathways. Furthermore, these results may not translate to the complex environment and interactions that occur during infection *in vivo*. The use of animal models also permits comparisons of isolates from primary and disseminated sites of infection, as well as isolates that are shed at different time points during infection, which can provide further insight into the genetic traits that are associated with enhanced pathogenicity. Serial passaging has the potential to uncover *novel* genetic and phenotypic markers that contribute to enhanced virulence. In contrast, because forward genetic screens involving random mutagenesis typically focus on regions that are suspected or known to play a role in phenotypes underlying pathogenicity, this approach can discover novel *genetic* markers for enhanced virulence only. The targeted genetic modification approach is limited to the investigation of genetic traits and underlying phenotypes that are suspected to contribute to pathogenicity (e.g., determining whether enhanced polymerase activity contributes to pathogenicity).

Targeted genetic modification is also used to confirm that particular mutations or gene segments are *necessary* and *sufficient* to enhance virulence in mammals. Often this experiment is followed by characterization of other virus phenotypes, such as infectivity and tissue tropism. Furthermore, this approach provides associative insight into how host responses are altered during infection with the modified strain. Collectively, this information provides a strong foundation for follow-up studies investigating the mechanistic basis of pathogenicity, including the study of host-virus interactions.

Taken together, these GoF studies provide a foundation for follow-up cell biological, immunological, and pathological studies that elucidate the mechanistic basis of viral factors contributing to virulence, corresponding host responses, and how both factors alter susceptibility to secondary bacterial infection. Additionally, this approach permits the identification of host immune responses that are associated with enhanced pathogenicity. Although the analysis of host factors contributing to enhanced pathogenicity is indirect, the information can be derived from the comparison of genetically similar virus backgrounds displaying a dynamic range of virulence (i.e., GoF and parental strains). The relevance of these

approaches depends on whether mechanisms underlying enhanced virulence in cell culture and animal models are representative of those in humans. This limitation may be particularly relevant for the interpretation of studies involving mice, which are commonly used for pathogenicity studies but display distinct pathogenesis and natural susceptibility to human influenza viruses. Alternatively, ferrets have similar susceptibility, tissue tropism, and clinical signs of disease in response to infection with influenza viruses as humans. Another drawback of these approaches is that results gleaned from the study of one or a few strains may not be recapitulated in different genetic contexts.

15.4.3.1.3 Potential Benefits and Limitations of Alt-GoF Approaches

Several alt-GoF approaches can be used to uncover genetic and phenotypic traits underlying pathogenicity in mammals. First, comparing the sequences of human isolates that display varying degrees of pathogenicity enables the identification of genetic changes that are associated with increased virulence. Unlike the GoF approaches described above, this approach has the potential to directly identify genetic traits that contribute to pathogenicity in humans and may be more likely to uncover conserved traits through analysis of a large number of strains. However, this approach is subject to significant limitations relative to GoF approaches. First, the success of this approach depends on the availability of a wide breadth of surveillance data accompanied by epidemiological data about the clinical severity and case fatality rates of particular strains or groups of strains. The fact there is considerable variability in the type and magnitude of immune responses within human populations due to inherent genetic diversity, as well as differences in previous exposure to influenza and vaccination status, complicates the interpretation of genetic surveillance data. Because disease pathology can be exacerbated by host and viral factors, high-quality "metadata" about relevant host features (e.g., age, vaccination status, etc.) is needed so that sequences can be appropriately "binned" into low- and high-virulence categories for comparison. This is important for both the identification of *viral* factors (e.g., the neurotropism observed during H5N1 infections) that may contribute to virulence as well as the identification of *host* factors associated with enhanced pathogenicity (e.g., the immunopathology observed during H5N1 infections). Often, such metadata is not provided, is incomplete, and/or is not available as quickly as genetic data in standard surveillance practices, resulting in this approach being unfeasible or delayed relative to GoF approaches. Second, the use of consensus sequences in standard surveillance practices may not be able to uncover genetic traits that are present at low frequencies in human populations. Finally, the extensive genetic diversity within circulating virus populations makes discerning distinct viral genetic traits that are likely to contribute to pathogenicity difficult. Namely, the "noise" associated with comparing the sequences of isolates from different patients obscures the discovery of relevant features that distinguish isolates of varying pathogenicity, which practically limits this approach to the investigation of traits or regions previously known to be important for pathogenicity. A variant of the surveillance-based approach involves corroboration of sequence data with immunopathological observations from autopsies, which provides an opportunity to identify host factors or genetic polymorphisms that are broadly associated with severe disease.[1508] In addition to the limitations described above, this approach is limited by the availability of autopsy data and is subject to the caveat that autopsies represent late stage, lethal disease, which may not be representative. Comparing the sequences of isolates within patients, over the course of infection and/or from different tissue sources, represents another surveillance-based approach for identifying genetic traits that contribute to pathogenicity. Specifically, comparing early and late isolates during prolonged disease and comparing isolates from the primary site of infection (i.e., the upper respiratory tract) and those from disseminated sites (i.e., lower respiratory tract), which are associated with increased virulence, enables the identification of adaptive mutations that enhance virulence. A strength of this approach is that the reduced viral genetic diversity observed within a single patient may enable the identification of novel genetic traits associated with virulence. However, such traits may not be relevant in a broader patient context due to existing diversity in human susceptibility. Moreover, this is

[1508] Everitt AR *et al* (2012) IFITM3 restricts the morbidity and mortality associated with influenza. *Nature* 484: 519-523

limited to the analysis of viral isolates from patients presenting with severe disease, which may bias findings towards traits associated with prolonged and late stage disease.

Phenotypic characterization of wild type viruses in appropriate cell culture or animal models is another alt-GoF approach that can be used to study mechanisms underlying pathogenicity in mammals. Specifically, comparing the sequences of wild type viruses with varied levels of fitness *in vitro* and pathogenicity *in vivo* enables the identification of genetic and phenotypic traits associated with increased virulence in representative cell culture or animal models, respectively. Similar to GoF approaches, this approach can also identify host response pathways that are associated with varying disease outcomes, including susceptibility to secondary infection. Notably, the information generated through use of cell culture systems is limited relative to that generated through animal experiments due to the simplicity of the host immune response *in vitro*. Because of the high genetic diversity among existing viral isolates phenotypic characterization is often limited to the analysis of known determinants of pathogenicity unless highly genetically similar strains are available. The use of *in vivo* models is restricted to the study of viruses that can productively infect representative animal model systems, which excludes some animal-origin viruses with low fitness. (Such strains are typically passaged in mice for adaptation prior to analysis of virulence, which represents a GoF approach.) As for the GoF approaches, genetic and phenotypic traits uncovered through this approach may not translate to humans.

Loss of Function (LoF) approaches, genetic screens that utilize random mutagenesis or targeted genetic modification to identify changes that attenuate fitness/virulence, can also provide information about genetic and phenotypic traits that contribute to pathogenicity. The screening approach has the potential to identify novel genetic traits associated with pathogenicity, while the targeted approach is used to confirm whether particular genetic traits are *necessary* for pathogenicity. This information complements that generated by GoF methods, but LoF approaches suffer from several limitations. First, because of the high mutation rate of influenza viruses, LoF mutations that attenuate pathogenicity may revert during the single round of passage that is needed to characterize the virulence of the mutants (which represents a selection step). Second, although in principle, LoF screens for mutations that attenuate virulence can be performed in an unbiased manner, characterizing the pathogenicity of a large panel of mutants in animals is labor-intensive and expensive. As a result, the use of this method may be practically limited to cell culture systems or the investigation viral phenotypes previously shown to be associated with pathogenicity. Third, because many mutations attenuate pathogenicity for trivial reasons, for example mutations that compromise viability, discovering traits that directly contribute to virulence in high pathogenicity strains relative to low pathogenicity strains may be difficult using a LoF approach. However, mechanistic insight into the role of non-essential virus proteins, such as PB1-F2, is feasible using this approach, and the roles of essential proteins such as NS1 can be studied through specific deletion of non-essential functional domains. Of note, the virulence of highly attenuated strains can still be assessed in immunocompromised mice that are susceptible to infection, in order to identify secondary functions that contribute to virulence, but with decreased mechanistic insight into pathogenicity.

The use of replication incompetent viruses provides another alternative method for the identification of genetic and phenotypic traits underlying pathogenicity.[1509] In these model systems, viral replication and immune evasion pathways, both of which contribute to pathogenicity *in vivo*, can be assessed in cell culture lines that are engineered to stably express an essential viral protein that is missing from the "replication-incompetent" virus strains used for infection. For example, the replacement of the PB2 gene with a GFP-expression construct that has the necessary flanking, non-coding, and packaging sequences

[1509] The use of this approach has been proposed during interviews with influenza researchers as a possible method, although the use of this approach for explicitly identifying genetic and phenotypic viral and host factors contributing to fitness and cell-specific immune evasion is currently limited.

from the viral genome can only replicate in cell lines that stably express exogenous PB2.[1510] The result is a virus that is biologically constrained to replication in that cell line. Several replication incompetent model systems have been made, although some suffer from poor maintenance of the foreign gene/gene segment (GFP) during virus packaging.[1511,1512] Using these systems, viruses can be serially passaged to identify novel adaptive mutations (and phenotypic changes) that are associated with phenotypes underlying pathogenicity. However, cell culture systems cannot provide information about the effect of identified genetic traits on global host responses, virus dissemination, and associated morbidity and mortality. Accordingly, *in vitro* results may not be recapitulated during *in vivo* infection, a limitation that further weakens the utility of this approach. An additional concern is that, due to epistasis, existing cell lines, which express viral proteins from a particular strain, may not be compatible with other virus strain gene segments (i.e., a cell line expressing PB2 from a lab-adapted virus such as PR8 may not be compatible with the other gene segments of an avian influenza virus). If so, it may be necessary to generate new constructs or cell lines, perhaps decreasing the efficiency of this approach. Further characterization and validation of this model system will alleviate this limitation.

Several *in vitro* virus-free methods can be used to investigate phenotypes underlying pathogenicity. Cell biological assays (e.g., measuring polymerase activity or IFN-β induction) and crystallographic resolution of the structures of viral protein interactions with other viral or host factors (e.g., virus-host protein-protein complexes) can provide insight into the mechanistic and biophysical basis of underlying phenotypes. Comparative sequence analysis of viral proteins with different phenotypic properties can then enable the identification of mutations that are associated with relevant phenotypic changes or provide insight into the molecular basis for virus-host interactions. Alternatively, forward genetic screens can be used to identify novel genetic traits that contribute to underlying phenotypes, while targeted modification of viral gene segments in isolation confirms the set of genetic changes that are necessary and sufficient to alter an underlying phenotype. Though the simplicity and relatively high-throughput nature of these methods renders them appealing as a screening approach for the discovery of novel genetic traits associated with pathogenicity, these approaches are inherently limited to the investigation of previously identified viral phenotypes. An additional drawback is that results gleaned from studying the behavior of a viral protein or phenotype in isolation may not be recapitulated in the context of the full virus or *in vivo*. Although fairly rapid phenotypic assays have been developed for the study of phenotypic traits known to be associated with pathogenicity, assays to study certain phenotypic traits may be unreliable or unavailable for future phenotypes of interest.

The use of *in silico* approaches to model the biophysical properties of viral proteins, virus-host, and virus-virus protein complexes can be used to evaluate mutations that may alter phenotypes underlying pathogenicity. For example, results from modeling the glycosylation patterns of seasonal and pandemic HA proteins can be used to predict the susceptibility of different HA molecules to neutralization and inhibition by host immune proteins (e.g., collectins).[1513,1514] Although this approach may provide insight into the biophysical basis of interactions underlying phenotypes of interest, the success of the approach is limited by the accuracy of existing models.

[1510] Ozawa M *et al* (2011) Replication-incompetent influenza A viruses that stably express a foreign gene. *The Journal of general virology* 92: 2879-2888

[1511] Martínez-Sobrido L *et al* (2010) Hemagglutinin-Pseudotyped Green Fluorescent Protein-Expressing Influenza Viruses for the Detection of Influenza Virus Neutralizing Antibodies. *J Virol* 84: 2157-2163

[1512] Rimmelzwaan GF *et al* (2011) Use of GFP-expressing influenza viruses for the detection of influenza virus A/H5N1 neutralizing antibodies. *Vaccine* 29: 3424-3430

[1513] Sun X *et al* (2013) N-linked glycosylation of the hemagglutinin protein influences virulence and antigenicity of the 1918 pandemic and seasonal H1N1 influenza A viruses. *Journal of virology* 87: 8756-8766

[1514] Job ER *et al* (2010) Pandemic H1N1 influenza A viruses are resistant to the antiviral activities of innate immune proteins of the collectin and pentraxin superfamilies. *Journal of immunology (Baltimore, Md : 1950)* 185: 4284-4291

Finally, because pathogenicity reflects virus-host interactions, several alt-GoF approaches focus on identifying and characterizing host factors that are associated with pathogenicity, which may provide indirect insight into viral mechanisms underlying virulence in representative animal models. The use of transcriptional (e.g., qRT-PCR, microarray) and translational (e.g., ELISA) expression profiling, as well as immunophenotyping (e.g., identifying the type and kinetics of immune cell recruitment) and histopathology, independently or in the context of the GoF and alt-GoF approaches discussed above, can identify host response pathways that change during infection and thus may play a role in pathogenicity. The use of genetically modified mouse lines (e.g., knockout mice) or pharmacological inhibitors to confirm the role of a particular protein, signaling pathway, or immune cell type in pathogenicity provides further insight into the role of host-virus interactions. The strength of these approaches is that they provide direct information about host factors involved in pathogenicity. However, the immune response to influenza viruses is poorly understood and quite complex, making it difficult to resolve the function of particular host proteins in the context of globally altered host factors and regulatory networks.

Given the complexity of the immune response to influenza viruses in animal models, a more targeted approach involves *in vitro* proteomic (e.g., mass spectrometry) and genomic screens (e.g., RNAi screen) utilizing both virus-free and cell culture-based infection systems to discover host factors that interact with virus proteins of interest or that are critical for underlying phenotypes such as viral replication and immune evasion. These approaches provide direct insight into host factors involved in viral fitness. However, the breadth of proteomic approaches is limited in that screens typically focus on a single viral protein, and both genomic and proteomic screens can identify host proteins that may not be functionally relevant or may play minor roles in the viral life cycle *in vivo*. Furthermore, *in vitro* systems do not effectively capture the complex host environment, so the function and importance of host factors identified and studied in cell culture may not be recapitulated *in vivo*. The use of virus free, *in vitro* systems is further limited to the analysis of viral phenotypes in isolation and may not be conserved in the context of the full viral life cycle.

A second type of alternative approach involves the use of attenuated viruses, as a risk mitigation strategy. Four types of attenuated viruses could be used for such studies: (1) reassortants with surface protein gene segments from seasonal influenza viruses, to which the general population has pre-existing immunity, (2) reassortants with lab-adapted viruses (e.g., PR8), (3) strains which have virulence factors altered or deleted (e.g., deletion of the multi-basic cleavage site in HPAI HA sequences), and (4) strains which have incorporated binding sites for microRNAs (miRNAs) that are expressed in humans but not an animal model of interest, and therefore are replication-competent in experimental animals but not humans (termed "molecular biocontainment").[1515] The use of reassortants with lab-adapted strains to identify viral determinants that are *necessary* and *sufficient* to enhance virulence in a low-pathogenicity background is possible, as many of these strains are well characterized and provide a large dynamic range for evaluating increases in virulence. Despite those advantages, the results gleaned through use of the first three types of attenuated viruses are subject to the caveat of epistasis. That is, because complex, multi-genic traits depend on genetic context, causative genetic and phenotypic traits that contribute to enhanced virulence in attenuated strains may not be recapitulated in the context of other wild type strains and interactions with other factors (not present in the attenuated strain) may contribute to virulence. Similarly, differences in disease pathogenesis relative to wild type viruses further compromise the relevance of results gained through the use of some attenuated strains. Several additional factors limit the range of information that can be generated using the risk mediation approach. First, seasonal reassortant strains can only be used to study the role of internal gene segments in pathogenicity, while lab-adapted reassortants are limited to the study of proteins donated by the wild type strain. Other types of attenuated strains, such as strains in which the multi-basic cleavage site has been deleted, may not be suitable for *in vivo* studies. For all of

[1515] Langlois RA *et al* (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844-847

these methods, the mechanism of attenuation may alter phenotypes underlying virulence in representative animal models compromising the relevancy of information gleaned from the use of the attenuated strain.

Finally, although the microRNA-based molecular biocontainment strategy is considered promising by the influenza research community, only one such strategy has been developed to date, which involves incorporation of miRNA target sites that permit replication in ferrets but restrict replication in humans and mice (i.e., miR-192 target sites). As mice and human-derived cell lines are important model systems for the study of mechanisms underlying pathogenicity, existing miRNA-based risk mitigation strategies are of limited utility for these studies. Furthermore, existing engineered strains have not been extensively characterized with respect to infection dynamics and pathogenesis in ferrets or permissive cell lines. Additional research is needed to determine whether and to what extent the engineered strains serve as functional proxies for their cognate WT strains in these model systems, before these strains can be widely used to probe scientific questions about virulence and disease pathogenesis. Of note, the identification of suitable miRNAs that are expressed in humans but not mice may permit the use of this strategy to conduct GoF studies that enhance virulence in mice in the future, thereby improving its broad utility.

15.4.3.1.4 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Tables 15.20 and 15.21 provide a summary of the benefits and limitations of GoF and alt-GoF approaches that can address scientific knowledge gaps about the mechanisms underlying viral virulence and disease pathogenesis in mammals. The underlying genetic and phenotypic features that result in infectivity, pathogenicity, and associated morbidity and mortality during influenza virus infection are poorly understood, in part because of the complex interplay between virus and host factors during pathogenesis. Many host and viral factors synergize to exacerbate pathology, making mechanisms difficult to tease apart. Considerable gaps in knowledge remain on the molecular basis and the role of each underlying viral phenotype in determining virulence. Moreover, there is limited knowledge on host factors that contribute to protective immunity and immunopathology. Insight into virus-host interactions is needed to advance in-depth understanding of virulence and pathogenesis of influenza viruses. By differentiating between virus and host factors contributing to pathogenesis, it may be possible to target viral factors that drive pathogenicity and to uncouple deleterious and protective effects of the immune response through host-targeted therapeutics. Because GoF and alt-GoF approaches have distinct benefits and limitations for the study of viral factors versus host factors that contribute to pathogenicity, their relative value for identifying and characterizing virus factors versus host factors is evaluated separately.

Identification and Characterization of Viral Factors That Contribute to Pathogenicity

The ability of GoF versus alternative approaches to provide insight into the viral factors governing virulence and disease pathogenesis is first summarized. Taken together, GoF approaches represent the most efficient and effective strategies for identifying novel viral genetic traits that contribute to the pathogenicity of any virus strain. In addition, targeted genetic modification of viruses to introduce traits associated with pathogenicity is uniquely capable of demonstrating that particular viral genetic traits are *necessary* and *sufficient* to enhance virulence across multiple virus contexts. However, results gleaned from cell culture and animal model studies may not translate to humans. Notably, the use of attenuated strains for these studies is hindered by the fact attenuation may alter disease pathogenesis, thus results may not be recapitulated in the genetic context of the wild type virus. In addition, attenuated strains cannot be used when the mechanism of attenuation alters the viral factor or underlying phenotype studied. However, the introduction of genetic traits associated with virulence to lab-adapted strains provides a controlled system for the dissection of the functions of individual genetic or phenotypic traits that contribute to virulence, and the fact that lab-adapted strains are attenuated permits investigation of a large spectrum of virulence. Although the newly developed microRNA-based molecular biocontainment strategy is considered promising by the influenza research community, the fact that existing strategies

restrict viral replication in humans and mice significantly limits the current utility of this strategy for pathogenicity studies, which often involve mice or human cell lines.

Although comparative sequence analysis of surveillance data has the potential to uncover viral genetic traits that are associated with virulence in humans, the utility of this approach is significantly compromised by shortcomings in the quality and availability of associated metadata, which are needed to control for variability in the human immune response and susceptibility to influenza viruses. Additionally, this approach is practically limited to the investigation of known viral genetic traits due to the high genetic diversity among influenza viruses. For the same reason, characterization of wild type isolates is limited to the study of previously known traits, unless genetically similar strains are available. In contrast, comparative analysis of isolates within patients enables the identification of novel adaptive traits that are associated with enhanced virulence over the course of infection. However, this approach is often biased to severe and late stage infection and is further complicated by the fact that individual genetic and environmental host factors impacting immunity, thus results may not be broadly conserved in human populations. LoF approaches also have limited utility for broad and unbiased identification of novel genetic and phenotypic traits due to their inefficiency, including the fact that LoF approaches may uncover traits that indirectly contribute to pathogenicity. Notably, targeted LoF enables the identification of genetic and phenotypic traits that are *necessary* for enhanced virulence, which provides valuable information to complement and strengthen results gleaned from targeted GoF studies.

While *in vitro*, virus free approaches and use of replication incompetent viruses enable the identification of novel genetic and phenotypic traits that are necessary and sufficient to alter phenotypes underlying pathogenicity, the importance of those genetic traits in the context of the complex host environment is difficult to extrapolate. Moreover, the *in vitro*, virus free and cell culture methods do not provide any information on mechanisms underlying the morbidity and mortality associated with influenza infection.

Finally, host-focused approaches provide indirect insight into the function of virus proteins and thus are of limited utility for understanding how viral factors contribute to pathogenicity, relative to GoF approaches.

Identification and Characterization of Host Factors That Contribute to Pathogenicity

Both GoF and alt-GoF approaches can provide insight into host factors that enhance pathogenicity, including deleterious immune responses that contribute to the morbidity and mortality caused by influenza infection. GoF approaches can be used to identify host factors that are *associated* with enhanced virulence, morbidity, and mortality. In particular, targeted genetic modification to introduce traits that are expected to enhance virulence provides a controlled system that can be used to tease apart the interplay between virus and host factors contributing to pathogenesis (i.e., by demonstrating how changes to a particular virus factor alter host immune responses and enhance infection-associated-pathology). The utility of using risk-mediation reassortants in lieu of wild type viruses is significantly limited for the study of host factors that contribute to pathogenicity. Pathogenicity is derived from the complex interplay between many underlying viral traits and host factors that are not fully captured in the context of different genetic backgrounds or when pathogenicity is severely impaired (e.g., with the use deletion of the MBCS). The main drawback of GoF approaches, with respect to the study of *host* factors that contribute to pathogenicity, is that they cannot establish a causal link between a host factor and enhanced pathogenicity and/or more severe disease pathology. Additionally, results from representative animal models may not translate to humans.

The use of targeted knockout animals or pharmacological inhibition of the host factor during infection, an alt-GoF approach, is uniquely capable of confirming that a host factor contributes to virulence and pathogenicity. However, because the host response is dynamic and complex, inhibition of a host factor is

likely to have a multi-faceted effect on immune responses during infection, making the identification of host traits that drive viral clearance and deleterious immune responses difficult to resolve. Targeted genetic modification of viruses to introduce traits expected to attenuate virulence (LoF) can also be used to identify host factors/responses that are associated with enhanced pathogenicity. Like its GoF counterpart (i.e., targeted genetic modification of viruses to introduce traits expected to enhance virulence), this approach provides a controlled system for studying interplay between virus and host factors contributing to pathogenesis, and the resulting information complements results from GoF studies. However, LoF approaches have limited utility for studying host proteins that interact with viral proteins that are required for fitness/infectivity. Immunological characterization of wild type isolates exhibiting varied levels of virulence can demonstrate an association between a particular host response and exacerbated disease pathology. However, this approach provides little mechanistic insight into the role of particular virus-host interactions if viral isolates display high genetic diversity. Several other alt-GoF approaches provide correlative data about the course of disease and the immune responses that are associated with severe outcomes observed in humans, including comparative analysis of genetic surveillance data, analysis of patient isolates, and analysis of autopsy data. This information is highly valuable for connecting results observed in animal model systems to nature (e.g., whether neurotropism observed during infections of ferrets with H5N1 viruses is representative of human infections). However, these approaches provide limited mechanistic insight and are impaired by limitations in the quality and availability of genetic surveillance data, in particular a lack of high quality metadata.

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
GoF #1a [1,4,5]*: Targeted genetic modification to introduce genetic changes expected to contribute to pathogenicity (*in vivo*)	• Identifies viral genetic and phenotypic traits that are **necessary** and **sufficient** for enhanced pathogenicity (i.e., provides causative data) • Identifies host factors **associated** with deleterious or protective outcomes • Gain insight into viral phenotypes underlying pathogenicity • Gain insight into host mechanisms underlying disease pathology • Controlled system for the study of how virus-host interactions contribute to pathogenicity • Proactive - can be performed using viruses that do not display enhanced pathogenicity in humans • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from representative animal models may not translate to mechanisms underlying pathogenicity in humans • Bias – Limited to investigation of previously identified phenotypic *or* genetic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved • Indirect insight into host factors contributing to pathogenicity

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
GoF #1b [1,4,5]: Targeted genetic modification to introduce genetic changes expected to contribute to fitness and immune evasion (*in vitro*)	• Identifies genetic and phenotypic traits that are **necessary** and **sufficient** for viral fitness (i.e., provides causative data) • Identifies host factors **associated** with phenotypes underlying fitness/immune evasion • Gain insight into viral phenotypes underlying fitness/immune evasion • Gain insight into host mechanisms underlying cell-specific immunity • Controlled system for the study of how virus-host interactions contribute to pathogenicity • Proactive - can be performed using viruses that do not display enhanced pathogenicity in humans • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Limited to the investigation of viral fitness and cell-specific immune evasion pathways, which are components of pathogenicity • Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from cell culture models may not translate to humans • Bias – Limited to investigation of previously identified phenotypic *or* genetic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved • Indirect insight into host factors contributing to fitness/immune evasion

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
GoF #2a [2]: Forward genetic screen to introduce genetic changes that may contribute to pathogenicity, followed by testing *in vivo*	• Identifies **novel** genetic traits that are sufficient for enhanced pathogenicity • Identifies host factors **associated** with deleterious or protective outcomes • Gain insight into viral phenotypes underlying pathogenicity • Gain insight into host mechanisms underlying disease pathology • Proactive - can be performed using viruses that do not display enhanced pathogenicity in humans	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from representative animal models may not translate to mechanisms underlying pathogenicity in humans • Bias – Limited to investigation of previously identified **phenotypic** traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • Associative – Information produced is correlative, not causative • Indirect insight into host factors contributing to pathogenicity
GoF #2b [2]: Forward genetic screen to introduce genetic changes that may contribute to phenotypes underlying pathogenicity, followed by testing *in vitro*	• Identifies novel genetic traits that are sufficient to enhance viral fitness/immune evasion • Identifies host factors **associated** with phenotypes underlying fitness/immune evasion • Gain insight into viral phenotypes underlying fitness/immune evasion • Gain insight into host mechanisms underlying cell-specific immunity • Proactive - can be performed using viruses that do not display enhanced pathogenicity in humans • *In vitro* methods can be used to screen a larger number of mutants than *in vivo* methods	• Narrow breadth – Results may not generalize to other virus strains • Limited to the investigation of viral fitness, which is one component of pathogenicity • Translatability – Results from cell culture models may not translate to humans • Bias – Limited to investigation of previously identified **phenotypic** traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • Associative – Information produced is correlative, not causative • Indirect insight into host factors contributing to fitness/immune evasion

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
GoF #3a [3]: Serial passaging with selection for pathogenicity, use of animal models (*in vivo*)	• Identifies novel genetic and phenotypic traits that are sufficient for enhanced pathogenicity • Identifies host factors **associated** with deleterious or protective outcomes • Gain insight into viral phenotypes underlying pathogenicity • Gain insight into host mechanisms underlying disease pathology • Proactive - can be performed using viruses that do not display enhanced pathogenicity in humans	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from representative animal models may not translate to mechanisms underlying pathogenicity in humans • Associative – Information produced is correlative, not causative • Indirect insight into host factors contributing to pathogenicity
GoF #3b [3]: Serial passaging with selection for fitness, use of cell culture models (*in vitro*)	• Identifies novel genetic and phenotypic traits that are sufficient to enhance viral fitness/immune evasion • Identifies host factors **associated** with phenotypes underlying fitness/immune evasion • Gain insight into viral phenotypes underlying fitness/immune evasion • Gain insight into host mechanisms underlying cell-specific immunity • Proactive - can be performed using viruses that do not display enhanced pathogenicity in humans	• Narrow breadth – Results may not generalize to other virus strains • Limited to the investigation of viral fitness and cell-specific immune evasion pathways, which are components of pathogenicity • Translatability – Results from cell culture models may not translate to humans • Associative – Information produced is correlative, not causative • Indirect insight into host factors contributing to fitness/immune evasion

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #1 [1]: Comparative sequence and epidemiological data analysis of human isolates	• Identifies genetic traits that are associated with enhanced pathogenicity o Comparison of genetically similar viruses can result in the identification of previously unknown genetic traits that are associated with enhanced pathogenicity o Depending on the size of analysis and strength of association some traits can be considered "causally" linked o **Directly** translates to **human** disease o Analyzes *broad* data sets applicable to many strains • Identifies host factors that **correlate** with deleterious or protective outcomes • Gain insight into the prevalence and distribution of genetic traits o Can infer functional generalizability of genetic traits (i.e., whether they do or do not behave similarly in different genetic contexts)	• Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • High diversity in host population o Variability in the type and magnitude of immune responses observed in human populations due to genetic diversity, vaccination history, and previous exposure to influenza • Limited by the quality and availability of existing surveillance data o Consensus sequences may not capture low frequency mutations o High-quality metadata on relevant host factors needed to appropriately bin groups for comparison may not be available, is incomplete, or is delayed relative to sequences o Limited reporting of negative surveillance data • Associative – Information produced is correlative, not causative • Reactive – Analysis of viral isolates that already exist in nature

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations

Alt-GoF #2 [2]:
Comparative sequence and clinical data analysis of human isolates from a single patient
	• Identifies genetic traits that are associated with enhanced pathogenicity
	  o Comparison of genetically related viruses can result in the identification of previously unknown genetic traits that are associated with enhanced pathogenicity
	  o **Directly** translates to **human** disease
	• Identifies host factors that **correlate** with deleterious or protective outcomes
		• Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important
		• Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty
		• Limited availability of case studies
		• Analysis is biased towards severe and often late stage disease
		• High diversity in host population
		  o Variability in the type and magnitude of immune responses observed in human populations due to genetic diversity, vaccination history, and previous exposure to influenza
		• Limited by the quality and availability of existing surveillance data
		  o Consensus sequences may not capture low frequency mutations
		  o High-quality metadata on relevant host factors needed to appropriately bin groups for comparison may not be available, is incomplete, or is delayed relative to sequences
		  o Limited reporting of negative surveillance data
		• Associative – Information produced is correlative, not causative
		• Reactive – Analysis of viral isolates that already exist in nature

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #3 [3]: Characterization of wild type viruses	• Identifies genetic and phenotypic traits that are **necessary** and **sufficient** for enhanced pathogenicity (i.e., provides causative data) ○ Comparison of genetically similar viruses can result in the identification of **sufficient** genetic and phenotypic traits • Identifies host factors **associated** with deleterious or protective outcomes • Gain insight into viral phenotypes underlying pathogenicity • Gain insight into host mechanisms underlying disease pathology	• Bias – High genetic diversity among influenza strains limits this approach to the examination of genetic regions already known to be important • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • Animal influenza viruses that have poor fitness in representative animal models provided limited insight • Genetic diversity of viral isolates limits the amount of in-depth mechanistic insight into the interplay between virus-host • Translatability – Results may not translate to mechanisms underlying pathogenicity in humans • Associative – Information produced is correlative, not causative • Reactive – Analysis of viral isolates that already exist in nature

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #4 [4]: LoF forward genetic screen to introduce genetic changes that may attenuate virulence, followed by testing in vitro or in vivo	• Identifies previously unknown genetic and phenotypic traits that are **necessary** for pathogenicity • Identifies host factors **associated** with deleterious or protective outcomes • Gain insight into viral phenotypes underlying pathogenicity • Gain insight into host mechanisms underlying disease pathology	• Translatability – Results may not translate to mechanisms underlying pathogenicity in humans • Narrow breadth – Results may not generalize to other virus strains • Attenuated virus may recover virulence during characterization • Bias – Limited to investigation of previously identified **phenotypic** traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • Triviality – May uncover mutations that indirectly attenuate pathogenicity, which provides limited mechanistic insight o Less of a concern if targeting specific domains or regions of the influenza genome which are not required for viability (e.g., NS1 functional domains) • Practically limited to the use of cell culture systems • Ethical considerations and resources required for animal experiments preclude screening of a large number of mutants *in vivo*

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #5 [5,14,19]: Targeted LoF to introduce genetic changes expected to attenuate pathogenicity	• Identifies genetic and phenotypic traits that are **necessary** for pathogenicity • Identifies host factors **associated** with deleterious or protective outcomes • Gain insight into viral phenotypes underlying pathogenicity • Gain insight into host mechanisms underlying disease pathology • Controlled system for the study of how virus-host interactions contribute to pathogenicity • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Narrow breadth – Results may not generalize to other virus strains • Attenuated virus may recover virulence during characterization • Bias – Limited to investigation of previously identified phenotypic *or* genetic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • Triviality – May uncover mutations that indirectly attenuate pathogenicity, which provides limited mechanistic insight o Less of a concern if targeting specific domains or regions of the influenza genome which are not required for viability (e.g., NS1 functional domains) • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #6 [6,15]: (*In vitro*, replication incompetent model system) Targeted genetic modification to introduce genetic changes expected to contribute to fitness and immune evasion	• Identifies genetic and phenotypic traits that are **necessary** and **sufficient** for viral fitness (i.e., provides causative data) • Gain insight into viral phenotypes underlying fitness/immune evasion • Proactive - can be performed using viruses/combinations of virus gene segments that do not display enhanced pathogenicity in humans • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Narrow breadth – Results may not generalize to other virus strains • Limited Utility – Replication incompetent systems have only been developed and validated for a limited number of strains o Use of existing models for other strains will depend on genetic compatibility • Bias – Limited to investigation of previously identified phenotypic *or* genetic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty
Alt-GoF #7 [7]: (*In vitro*, replication incompetent model system) Serial passaging with selection for fitness	• Identifies novel genetic and phenotypic traits that are **necessary** for viral fitness (i.e., provides causative data) • Gain insight into viral phenotypes underlying fitness/immune evasion • Proactive - can be performed using viruses/combinations of virus gene segments that do not display enhanced pathogenicity in humans	• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Narrow breadth – Results may not generalize to other virus strains • Associative – Information produced is correlative, not causative • Limited Utility – Replication incompetent systems have only been developed and validated for a limited number of strains o Use of existing models for other strains will depend on genetic compatibility

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #8 [8]: (*In vitro*, virus-free) Forward genetic screen to introduce genetic changes that may alter phenotypes underlying fitness	• Identifies novel genetic traits that are sufficient to alter phenotypes underlying fitness • Provides insight into mechanistic basis of underlying phenotypes • *In vitro* methods can be used to screen a larger number of mutants than *in vivo* methods • Proactive - can be performed on virus gene segments from viruses that do not display enhanced pathogenicity in humans	• Simplicity of model system – Results based on the study of a viral protein/phenotype in isolation may not be recapitulated in the context of the full virus • Narrow breadth – Results may not generalize to other virus strains • Bias – Limited to investigation of previously identified phenotypic traits • Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty • State of methodology – Relies upon phenotypic assays, which may be unreliable or unavailable • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved
Alt-GoF #9 [16,20]: (*In vitro*, virus-free) Targeted genetic modification to introduce genetic changes expected to alter phenotypes underlying fitness	• Identifies genetic traits that are **necessary** and **sufficient** to alter a phenotype underlying fitness • Provides insight into the mechanistic basis of phenotypes underlying fitness • Proactive - can be performed on virus gene segments from viruses that do not display enhanced pathogenicity in humans • Enables testing of markers in different viral gene segments to assess generalizability of previous findings	
Alt-GoF #10 [9]: (*In vitro*, virus-free) Structural studies to analyze the molecular basis of fitness/immune evasion	• Provides insight into biophysical mechanisms underlying virus-host and virus-virus protein interactions contributing to fitness o Provides detailed mechanistic information • Proactive - can be performed using *select* virus gene segments from viruses that do not display enhanced pathogenicity in humans depending on the state of methodology	

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #11 [17,21]: (*In silico*, virus-free) Modeling to analyze the biophysical effects of mutations contributing to pathogenicity	• Provides insight into biophysical mechanisms underlying virus-host and virus-virus protein interactions • Provides detailed mechanistic information • Proactive - can be performed on virus gene segments from viruses that do not display enhanced pathogenicity in humans • Enables prediction of phenotypic consequences of markers in different viral gene segments to assess generalizability of previous findings	• Predictive – Does not confirm or correlate phenotypic effects in a biological context • Simplicity of model system – Results based on the study of a viral protein/phenotype in isolation may not be recapitulated in the context of the full virus • Model accuracy – Utility of the approach depends on the quality of existing models

Alt-GoF #12 [10][c]:
Proteomic screen to identify host proteins that physically interact with viral proteins during infection
	• Identifies host proteins that **may** play a role in fitness **during infection**
	  o Reveals **previously unknown** host factors
	  o Reveals **previously unknown** host-virus interactions during infection
	• Provides insight into the role of particular virus-host interactions **during infection**
		• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans
		• Narrow breadth – Results may not generalize to other virus strains
		• Bias – Limited to investigation of previously identified phenotypic traits
		• Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty
		• Identified host protein may not be functionally relevant or may play a minor role in viral life cycle
		• Indirect – Identification of host proteins or virus-host interactions that contribute to adaptation/transmissibility provides indirect information about viral genetic and phenotypic traits underlying adaptation
		  o Mechanistic insight may depend on prior knowledge of virus-host interactions

Alt-GoF #13 [11]: Genomic screen to identify host factors that contribute to fitness		

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations

Alt-GoF #14 [13]:
(*In vitro*, virus-free) Proteomic or genomic screen to identify host factors that interact with particular virus proteins and/or contribute to fitness
	• Identifies host proteins that **may** play a role in fitness
	  ○ Reveals **previously unknown** host factors contributing to underlying phenotypes
	  ○ Reveals **previously unknown** host-virus interactions contributing to underlying phenotypes
	• Provides direct insight into the role of particular virus-host interactions
	• Proactive - can be performed on virus gene segments from viruses that do not display enhanced pathogenicity in humans
		• Narrow breadth – Results may not generalize to other virus strains
		• Bias – Limited to investigation of previously identified phenotypic traits
		• Limited/poor foundational knowledge of the linkage between underlying phenotypes and pathogenicity and the interplay among and between viral and host factors provide further uncertainty
		• Identified host protein may not be functionally relevant or may play a minor role in viral life cycle
		• Indirect – Identification of host proteins or virus-host interactions that contribute to adaptation/transmissibility provides indirect information about viral genetic and phenotypic traits underlying adaptation
		  ○ Mechanistic insight may depend on prior knowledge of virus-host interactions
		• Simplicity of model system – Results based on the study of a viral protein/phenotype in isolation may not be recapitulated in the context of the full virus

Table 15.20. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits— What Are the Genetic and Phenotypic Traits of Pathogenicity of Influenza Viruses? What Are the Host Factors Associated with Pathogenicity and Influenza Associated Morbidity/Mortality?

Experimental Approach	Benefits	Limitations
Alt-GoF #15 [12,22]: Targeted modification of host factor to alter expression or function of host factors expected to contribute to pathogenicity	• Identifies and confirms host factors that contribute to deleterious or protective outcomes • Gain insight into viral phenotypes underlying pathogenicity • Gain direct insight into host mechanisms underlying disease pathology • Enables testing of the role of host markers in pathogenicity in the context of infection with new viral strains, to assess generalizability of previous findings	• Translatability – Results may not translate to mechanisms underlying fitness/transmissibility in humans • Narrow breadth – Results may not generalize to other virus strains • Identified host protein may not be functionally relevant or may play a minor role in viral life cycle • May be difficult to resolve function of host protein in the context of global alteration of the host protein • Indirect – Identification of host proteins or virus-host interactions that contribute to adaptation/transmissibility provides indirect information about viral genetic and phenotypic traits underlying pathogenicity • Mechanistic insight may depend on prior knowledge of virus-host interactions • Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved

[a] *GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets reference the order in the landscape tables (Supplementary Information).*

Table 15.21. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity		
Scientific Knowledge Benefits – Utility and Limitations of Using Attenuated Strains for GoF Approaches That Enhance Pathogenicity		
Virus[a]	**Limitations**	**Experimental System[b]**
High Pathogenicity Strain[c] • Animal strain • Pathogenic reassortant	**N/A**	*In vivo* • Virulence studies (*the virus would likely be functional and representative of wild type conditions in vitro and in vivo*) *In vitro* • Characterization of underlying phenotypes of pathogenicity (*the virus would likely be functional and representative of wild type conditions in vitro and in vivo*)
Risk mediation Reassortant-Seasonal influenza	**Genetic Context** • Complex phenotypes are multi-genic; results may not be recapitulated in the context of the wild type virus **Limited Utility** • **Precludes study of the role of animal-origin HA and NA proteins,** which are critical viral factors in pathogenicity **Overlapping phenotypes** • Method of attenuation may alter phenotypes that contribute to virulence, thus interfering with the study of virulence **Altered course of disease** • Animals infected with attenuated viruses may exhibit significantly different disease pathology, limiting the relevance of outcomes to wildtype viruses	*In vivo* • Virulence studies (*the virus may not be functional or representative in vitro or in vivo*) *In vitro* • Characterization of underlying phenotypes of pathogenicity (*the virus may not be functional or representative in vitro or in vivo*)

Table 15.21. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity

Scientific Knowledge Benefits – Utility and Limitations of Using Attenuated Strains for GoF Approaches That Enhance Pathogenicity

Virus[a]	Limitations	Experimental System[b]
Risk mediation Reassortant-Lab-adapted (e.g., PR8)	**Limited model systems** • Lab-adapted strains are not highly infectious in ferrets **Genetic Context** • Complex phenotypes are multi-genic; results may not be recapitulated in the context of the pathogenic virus **Overlapping phenotypes** • Method of attenuation may alter phenotypes that contribute to virulence, thus interfering with the study of virulence **Altered course of disease** • Animals infected with attenuated viruses may exhibit significantly different disease pathology, limiting the relevance of outcomes to wildtype viruses	*In vivo* • Virulence studies (*the virus may not be functional or representative in vitro or in vivo*) *In vivo* • Characterization of underlying phenotypes of mammalian adaptation and transmissibility (*the virus may not be functional or representative in vitro or in vivo*)
Attenuated Strain • Targeted mutagenesis to remove virulence factor (e.g., ΔMBCS)	**Genetic Context** • Complex phenotypes are multi-genic; results may not be recapitulated in the context of the wild type virus **Overlapping phenotypes** • Method of attenuation may alter phenotypes that contribute to virulence, thus interfering with the study of virulence	*In vivo* • Virulence studies (*the virus would likely be non-functional in vitro or in vivo*) *In vivo* • Characterization of underlying phenotypes of pathogenicity (*the virus would likely be functional and representative of wild type conditions in vitro and in vivo*)
Molecular Biocontainment • Incorporation of binding sites for miRNAs expressed in humans but not experimental animals	**Limited model systems:** • Engineered strains to date are capable of replicating in ferrets but not mice or humans, which limits the model systems that can be used for *in vivo* and *in vitro* studies[d] • Strategy has been validated in two strains only **Potential for Altered Virus Function** • Whether incorporation of miRNA target sites alters the biology of the virus, including viral pathogenesis, has not yet been extensively characterized	*In vivo* • Virulence studies in ferrets[c] (*the virus may not be functional or representative in vitro or in vivo*) *In vitro* • Characterization of underlying phenotypes pathogenicity using cells that do not express miR-192 (excludes human and mice cell lines)[e] (*the virus would likely be non-functional in vitro or in vivo*)

Table 15.21. Summary of the Benefits of GoF Approaches That Enhance Pathogenicity		
Scientific Knowledge Benefits – Utility and Limitations of Using Attenuated Strains for GoF Approaches That Enhance Pathogenicity		
Virus[a]	**Limitations**	**Experimental System[b]**

[a] Animal-origin strains include avian- and swine-origin strains that have and have not infected humans. Pandemic strains include the 1918 H1N1, 1957 H2N2, and 1968 H3N2 viruses. Seasonal strains include all seasonal isolates and 2009 H1N1 pandemic isolates (now circulating seasonally). Risk mediation reassortants include all reassortants with lab-adapted viruses or with surface protein gene segments from seasonal influenza viruses. Pathogenic reassortants include viruses with animal and/or human gene segments (both seasonal and pandemic) for which human populations have limited or no immunity.

[b] The text color in the experimental system column indicates the general feasibility of the use of the virus described for in vivo or in vitro use. Green indicates that the virus would likely be functional and representative of wild type conditions in vitro and in vivo, orange indicates that the virus may not be functional or representative in vitro or in vivo, and red indicates that the virus would likely be non-functional in vitro or in vivo.

[c] GoF approaches are shaded in blue, and alt-GoF approaches (i.e., conducting GoF approaches using attenuated strains in lieu of wild type strains) are shaded in grey.

[d] Langlois et al. incorporated target sites for miR-192, which is expressed in humans and mice but not ferrets, into the HA genome segment of two different influenza A strains, thereby generating an engineered strain that is replication-competent in ferrets but not humans or mice.[1515,16]

[e] Assessment of suitable experimental systems reflects miRNA-based molecular biocontainment strategies published to date, i.e., the use of miR-192 target sites by Langlois et al.

[1515] Langlois RA *et al* (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844-847

15.4.3.2 Scientific Knowledge Gap 2: Discover Whether Fitness Defects Associated with the Acquisition of Antiviral Resistance Can Be Overcome, and the Mechanisms Underlying Recovery of Fitness

Though influenza viruses can readily mutate to acquire resistance to therapeutics, antiviral-resistant viruses are often initially less fit than parental viruses.[1517] For example, when the H274Y mutation in the NA gene (N1 numbering), which confers strong resistance to oseltamivir, first arose in nature, viruses carrying that mutation were less fit than their wild type counterparts.[1518] The relative fitness of antiviral-resistant strains has implications for how likely and how quickly these strains are to spread in nature. Whether and how antiviral strains can acquire compensatory mutations that enhance fitness while preserving the antiviral resistance phenotype is unknown for most antiviral resistance mutations. Studies investigating this question provide insight into the mechanistic basis of viral fitness and the mechanistic interplay between antiviral resistance and other virus phenotypes, and also are of interest for public health.

15.4.3.2.1 Benefits and Limitations of GoF Approaches

Several GoF approaches can be used to determine whether antiviral-resistance strains with impaired growth can recover fitness and to identify compensatory mutations that rescue growth, which provides a foundation for follow-up biochemical and cell biological studies that investigate the mechanistic basis of enhanced growth. First, growth-impaired strains can be serially passaged in cells or animals to select for strains with enhanced fitness, following by sequencing of emergent viruses to identify genetic changes that arose. However, this approach often results in reversion of antiviral-resistance mutations rather than the evolution of compensatory mutations. A second approach involves forward genetic screens to identify mutations that are sufficient to rescue fitness. While this approach is more likely to uncover compensatory mutations than serial passaging, screening large libraries of mutants is relatively labor-intensive, particularly if mutations are introduced into multiple virus proteins (as compensatory mutations may arise in proteins that do not contain antiviral-resistance mutations). Finally, targeted mutagenesis is used to confirm that a particular mutation or set of mutations is necessary and sufficient to rescue the fitness of a growth-impaired strain.

15.4.3.2.2 Benefits and Limitations of Alt-GoF Approaches

Two alt-GoF approaches can be used to identify compensatory mutations that may rescue the growth of antiviral-resistant strains with impaired fitness. First, comparative analysis of the sequences of antiviral-resistant strains with varying levels of fitness may enable the identification of mutations that are *associated* with enhanced fitness. However, due to the high genetic diversity among influenza viruses, generating strong hypotheses about mutations that are linked to the recovery of fitness is difficult. In addition, this approach is reactive, limited to the discovery of compensatory mutations after antiviral-resistant strains have recovered growth in nature. A second approach involves computational modeling to predict mutations that may rescue the fitness of growth-impaired strains. While this approach has been used to successfully predict mutations that enhance the growth of antiviral-resistant strains carrying the

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[1517] Baek YH *et al* (2015) Profiling and characterization of influenza virus N1 strains potentially resistant to multiple neuraminidase inhibitors. *Journal of virology* 89: 287-299

[1518] Gubareva LV *et al* (2001) Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *J Infect Dis* 183: 523-531

H274Y mutation in NA (N1 numbering),[1519,1520] all predictions must be experimentally confirmed using targeted mutagenesis, a GoF approach. Additionally, because existing computational models cannot predict epistasis effects, the *in silico* approach is limited to the discovery of compensatory mutations that arise in the same protein carrying the antiviral-resistance mutations.

15.4.3.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The strengths and limitations of GoF and alt-GoF approaches that can be used to investigate whether and how antiviral-resistant strains can overcome fitness defects are summarized in Table 15.22. Taken together, GoF approaches are uniquely capable of proactively discovering compensatory mutations that rescue the fitness of any antiviral-resistant strain with impaired growth, as well as establishing a causal link between compensatory mutations and enhanced fitness. Computational modeling can be used to generate hypotheses about mutations that may rescue growth, but all predictions must be experimentally confirmed using GoF approaches. Comparative sequence analysis of antiviral-resistant strains with varied levels of fitness has significant limitations relative to other approaches.

[1519] Bloom JD, Glassman MJ (2009) Inferring Stabilizing Mutations from Protein Phylogenies: Application to Influenza Hemagglutinin. *PLoS Comput Biol* 5

[1520] Bloom JD *et al* (2010) Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. *Science (New York, NY)* 328: 1272-1275

Table 15.22. Summary of the Benefits of GoF Approaches That Enhance Virulence in Mammals

Scientific Knowledge Benefits – Discover Whether Fitness Defects Associated with the Acquisition of Antiviral Resistance Can Be Overcome, and the Mechanisms Underlying Recovery of Fitness

Approach	Benefits	Limitations
GoF #1 [3]*: Serial passaging of attenuated viruses in cells or animals	• Identifies novel compensatory mutations that are sufficient to rescue the growth of attenuated strains • Proactive – can be performed on any virus strain	• Often results in reversion of antiviral-resistance or other attenuating mutations rather than selection for compensatory mutations • Associative - Information produced is correlative, not causative
GoF #2 [2]: Forward genetic screen to identify compensatory mutations that rescue fitness	• Identifies novel compensatory mutations that are sufficient to rescue the growth of attenuated strains • Proactive – can be performed on any virus strain	• Screening large libraries of mutant viruses is labor-intensive • Information produced may be correlative, not causative
GoF #3 [1,4,5]: Targeted mutagenesis to introduce compensatory mutations expected to enhance the growth of attenuated strains	• Identifies compensatory mutations that are **necessary** and **sufficient** to rescue the growth of attenuated strains across multiple strain contexts • Gain insight into mechanisms underlying the recovery of fitness of antiviral-resistant strains • Proactive – can be performed on any virus strain	• Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved
Alt-GoF #1 [1]: Comparative sequence analysis of antiviral-resistant strains with varying levels of fitness	• Identifies compensatory mutations that are *associated* with enhanced growth of antiviral resistant strains with attenuated fitness	• Associative - Information produced is correlative, not causative • Challenging due to the high genetic diversity among influenza viruses • Reactive – limited to discovering compensatory mutations after antiviral-resistant strains have recovered growth in nature

Table 15.22. Summary of the Benefits of GoF Approaches That Enhance Virulence in Mammals

Scientific Knowledge Benefits – Discover Whether Fitness Defects Associated with the Acquisition of Antiviral Resistance Can Be Overcome, and the Mechanisms Underlying Recovery of Fitness

Approach	Benefits	Limitations
Alt-GoF #2 [9,17]: Computational modeling to predict compensatory mutations that will rescue the growth of antiviral-resistant strains with impaired fitness	• Predicts compensatory mutations that may rescue the growth of antiviral resistant strains with attenuated fitness • Proactive – can be performed on any virus strain	• Predictive – Does not confirm or correlate phenotypic effects in a biological context • Model accuracy – utility of approach depends on the quality of existing models • Limited to the prediction of compensatory mutations within antiviral target protein • Existing models cannot account for epistasis effects

**Numbers in brackets reference specific experimental approaches in the landscape tables (Supplementary Information).*

15.4.3.3 Scientific Knowledge and Vaccine/Therapeutic Development Benefit: Development of Animal Models

Model systems that can be efficiently infected by influenza viruses and exhibit the spectrum of disease observed during human infections are essential for the study of influenza-associated morbidity/mortality and for testing the safety and efficacy of new vaccines and therapeutics. Mice, a common animal model used for the development of influenza MCMs, are naturally resistant to infection with many influenza viruses. GoF or alt-GoF approaches can be used to develop animal models to study the effectiveness of MCMs against these viruses. The development of MCMs that protect against severe disease necessitates testing the efficacy of candidate MCMs in animal models that exhibit exacerbated disease pathology. In cases where wild type viruses cause a limited spectrum of disease, GoF or alt-GoF approaches may be used to generate model systems that display a larger dynamic range of virulence.

15.4.3.3.1 Potential Benefits and Limitations of GoF approaches

Serial passaging of influenza viruses in laboratory animals to generate animal models is performed for two purposes. The first purpose is the generation of viruses that are capable of efficiently infecting mice for the study of influenza pathogenesis and medical countermeasure (MCM) development, as mice are inherently resistant to infection with human seasonal influenza viruses and some animal influenza viruses. Mouse-adapted influenza viruses have been used extensively for pathogenesis studies and for testing the efficacy of candidate vaccines and therapeutics against seasonal and pandemic influenza viruses.[1521,1522,1523,1524,1525,1526,1527,1528,1529,1530,1531] The second purpose is the generation of viruses with enhanced pathogenicity to support the development of MCMs that are capable of protecting against severe disease.[1532] For example, this approach would facilitate testing of the protective efficacy of the stockpiled H5N1 vaccines against the H5N2 HPAI viruses that caused widespread outbreaks in domestic poultry populations in the spring of 2014 and continue to circulate in wild birds with sporadic spread to poultry, which is of interest to HHS. However, North American isolates of H5N2 are of low virulence in ferrets, and therefore cannot be used to reliably evaluate vaccine effectiveness. Limited passaging of an avian H5N2 isolate in ferrets would select for a virus with enhanced virulence in mammals, which would provide a more relevant assessment of the ability of the vaccine to protect against H5N2 infections in

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[1521] Bahgat MM *et al* (2011) Inhibition of lung serine proteases in mice: a potentially new approach to control influenza infection. *Virology journal* 8: 27

[1522] Sun K *et al* (2011) Seasonal FluMist vaccination induces cross-reactive T cell immunity against H1N1 (2009) influenza and secondary bacterial infections. *Journal of immunology (Baltimore, Md : 1950)* 186: 987-993

[1523] Droebner K *et al* (2011) Antiviral activity of the MEK-inhibitor U0126 against pandemic H1N1v and highly pathogenic avian influenza virus in vitro and in vivo. *Antiviral research* 92: 195-203

[1524] Kashyap AK *et al* (2010) Protection from the 2009 H1N1 pandemic influenza by an antibody from combinatorial survivor-based libraries. *PLoS pathogens* 6: e1000990

[1525] Leneva IA *et al* (2000) The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/Hong Kong/156/97 (H5N1) and A/Hong Kong/1074/99 (H9N2) influenza viruses. *Antiviral research* 48: 101-115

[1526] Govorkova EA *et al* (2001) Comparison of efficacies of RWJ-270201, zanamivir, and oseltamivir against H5N1, H9N2, and other avian influenza viruses. *Antimicrobial agents and chemotherapy* 45: 2723-2732

[1527] Ekiert DC *et al* (2011) A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science (New York, NY)* 333: 843-850

[1528] Moseley CE *et al* (2010) Peroxisome proliferator-activated receptor and AMP-activated protein kinase agonists protect against lethal influenza virus challenge in mice. *Influenza and other respiratory viruses* 4: 307-311

[1529] Tharakaraman K *et al* (2015) A broadly neutralizing human monoclonal antibody is effective against H7N9. *Proceedings of the National Academy of Sciences of the United States of America* 112: 10890-10895

[1530] Boon AC *et al* (2009) Host genetic variation affects resistance to infection with a highly pathogenic H5N1 influenza A virus in mice. *Journal of virology* 83: 10417-10426

[1531] Srivastava B *et al* (2009) Host genetic background strongly influences the response to influenza a virus infections. *PloS one* 4: e4857

[1532] (2015h) Interviews with influenza researchers.

humans (which are expected to be accompanied by mammalian adaptive mutations in the virus that enhance its virulence in humans).[1533,1534] One key strength of the GoF approach is that the use of genetically similar viruses that display a large range and magnitude of virulence represents a controlled system for comparing the effectiveness of vaccines and therapeutics against low and high pathogenicity viruses, which enables triaging of similar MCM candidates based on their ability to protect against severe disease. The main drawback associated with these approaches is that the passaging needed to adapt the virus to representative animal models may alter its phenotypic properties in ways that affect pathogenesis mechanisms and its susceptibility to MCMs under study, which may render findings misrepresentative.

15.4.3.3.2 Potential Benefits and Limitations of Alt-GoF Approaches

One alternative to the use of serially passaged viruses involves sensitization of the host to influenza virus infection. This involves increasing host susceptibility to infection through the use of inbred mouse lines, knockout/transgenic mice, or the treatment of mice or ferrets with immunosuppressants.[1535,1536,1537,1538,1539] This approach can enable the study of wild type viruses that do not efficiently infect wild type mice. For example, although BALC/c mice are resistant to infection with many influenza viruses, the inbred DBA.2 mouse line is susceptible to infection with a variety of influenza viruses and has been used to demonstrate the efficacy of vaccines and therapeutics against seasonal, pandemic, and animal influenza viruses, as well as to study pathogenesis mechanisms.[1540,1541,1542,1543,1544] A strength of this approach is that the generation of genetically similar hosts (or genetically identical hosts, if immunosuppressants are used) that display a range of disease outcomes provides a controlled system for comparing pathogenesis mechanisms and the effectiveness of MCM candidates to protect against more severe disease. The use of genetic modification is largely limited to the use of the mouse model system, for which there are a broad array of well-established tools. However, the mouse model is less representative of human disease than other animal models, such as the ferret. The use of immunosuppressants is a promising alternative. The key drawback of this approach is that results gleaned from the use of immunocompromised hosts may not translate to disease in healthy hosts.

A second alternative approach involves infection of wild type hosts with wild type viruses. As mice are naturally resistant to infection with many influenza viruses, the utility of this approach is limited for the

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1533 (2015o) Interview with U.S. government representative involved in influenza vaccine development.

1534 Pulit-Penaloza JA *et al* (2015) Pathogenesis and Transmission of Novel Highly Pathogenic Avian Influenza H5N2 and H5N8 Viruses in Ferrets and Mice. *Journal of virology* 89: 10286-10293

1535 Pica N *et al* (2011) The DBA.2 mouse is susceptible to disease following infection with a broad, but limited, range of influenza A and B viruses. Ibid. 85: 12825-12829

1536 Dengler L *et al* (2012) Immunization with live virus vaccine protects highly susceptible DBA/2J mice from lethal influenza A H1N1 infection. *Virology journal* 9: 212

1537 Kim JI *et al* (2013) DBA/2 mouse as an animal model for anti-influenza drug efficacy evaluation. *Journal of microbiology (Seoul, Korea)* 51: 866-871

1538 van der Vries E *et al* (2013) Prolonged influenza virus shedding and emergence of antiviral resistance in immunocompromised patients and ferrets. *PLoS pathogens* 9: e1003343

1539 Belser JA *et al* (2011) The ferret as a model organism to study influenza A virus infection. *Disease models & mechanisms* 4: 575-579

1540 Pica N *et al* (2011) The DBA.2 mouse is susceptible to disease following infection with a broad, but limited, range of influenza A and B viruses. *Journal of virology* 85: 12825-12829

1541 Dengler L *et al* (2012) Immunization with live virus vaccine protects highly susceptible DBA/2J mice from lethal influenza A H1N1 infection. *Virology journal* 9: 212

1542 Tharakaraman K *et al* (2015) A broadly neutralizing human monoclonal antibody is effective against H7N9. *Proceedings of the National Academy of Sciences of the United States of America* 112: 10890-10895

1543 Boon AC *et al* (2009) Host genetic variation affects resistance to infection with a highly pathogenic H5N1 influenza A virus in mice. *Journal of virology* 83: 10417-10426

1544 Srivastava B *et al* (2009) Host genetic background strongly influences the response to influenza a virus infections. *PloS one* 4: e4857

mouse model system. [1545] Ferrets are naturally susceptible to a broader range of wild type influenza viruses. The strength of this approach is that the use of wild type viruses and wild type hosts is more relevant to human disease than other model systems. However, wild type viruses may display a limited range of virulence, which limits their utility for this purpose. Moreover, the high genetic diversity among influenza viruses complicates the comparison of results from the use of two genetically diverse wild type strains that exhibit varying levels of pathogenicity.

15.4.3.3.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Model systems that can be efficiently infected by influenza viruses and exhibit the spectrum of disease observed during human infections are essential for the study of influenza-associated morbidity/mortality and for testing the safety and efficacy of new vaccines and therapeutics. The strengths and limitations of model systems that can be used to study influenza virus infection are summarized in Table 15.23. Although the ability to infect wild type hosts with wild type viruses would be ideal for broad translation of results to human populations, mice are naturally resistant to infection with many influenza viruses, and/or wild type viruses may display a limited spectrum of disease in mice and ferrets. In these cases, because pathogenicity and disease outcome is dependent on the interplay between virus and host, both GoF and alt-GoF approaches enable the development of model systems that expand the dynamic range of pathogenesis that is observed when using wild type viruses and wild type hosts. GoF approaches achieve this goal by enhancing the virulence of the virus through serial passaging, while alt-GoF approaches enhance host susceptibility to disease through targeted genetic modification or the use of immunosuppressants. Both approaches provide a controlled system for comparing the effectiveness of MCM candidates to protect against more severe disease, and both have limitations. Serial passaging (GoF) may change the phenotypic properties of the virus in ways that alter its susceptibility to the MCM in development, which would lead to misrepresentative findings. Modification of the host (alt-GoF) may alter host immune responses that are involved in the mechanism of action of the vaccine or therapeutic, complicating translation of findings to disease in healthy hosts. The genetic modification approach is limited to mice, although the use of immunosuppressants represents a promising approach for ferrets, which are better representative of human disease. Given these caveats, the use of model systems derived from GoF and alt-GoF approaches strengthens the validity of any findings.

[1545] Margine I, Krammer F (2014) Animal models for influenza viruses: implications for universal vaccine development. Pathogens (Basel, Switzerland) 3: 845-874.

Table 15.23. Summary of the Benefits of GoF Approaches That Enhance Virulence in Mammals

Benefits to Scientific Knowledge and MCM Development – Develop Animal Models for the Study of Influenza Pathogenesis and to Support MCM Development

Approach	Benefits	Limitations
GoF: Adapt virus to host: serially passage virus in host (mouse or ferret) to increase infectivity and virulence in that host	• Generate influenza viruses that efficiently infect wild type mice for the study of influenza pathogenesis and to support MCM development • Generate genetically similar viruses that exhibit a large range of virulence in ferrets, which provides a controlled system for evaluating the efficacy of MCM candidates to protect against severe disease	• Adaptive mutations may alter the biology of the virus and/or may alter susceptibility of the virus to MCMs, relative to the wild type virus
Alt-GoF #1: Sensitize host to influenza virus infection: use of inbred mouse lines or targeted knockout/transgenic mice	• Enables the study of wild type viruses • Generate genetically similar hosts that exhibit a large range of disease severity in response to infection, which provides a controlled system for evaluating the efficacy of MCM candidates to protect against severe disease	• Results in immunocompromised hosts may not translate to healthy populations • Limited to mice, which are less representative of human disease than ferrets ○ There are limited tools for genetic modification of ferrets
Alt-GoF #2: Sensitize host to influenza virus infection: treat host with immunosuppressants	• Enables the study of wild type viruses • Generate genetically similar hosts that exhibit a large range of disease severity in response to infection, which provides a controlled system for evaluating the efficacy of MCM candidates to protect against severe disease	• Results in immunocompromised hosts may not translate to healthy populations
Alt-GoF #3: Infection of wild type hosts with wild type viruses	• Results using wild type viruses and wild type hosts are most likely to broadly translate to human populations	• Mice are resistant to infection with many influenza viruses • Wild type viruses may display a limited range of virulence in naturally susceptible hosts • Genetic diversity between wild type isolates with naturally varying levels virulence complicates comparison of results

15.4.4 Benefits of GoF to Surveillance

One major goal of influenza surveillance is to monitor the evolution of circulating animal influenza viruses, in order to identify those viruses that pose a risk of emerging in human populations to cause a pandemic. Resources can then be dedicated to mitigating the risk factors associated with virus emergence and to preparing for a potential emergence event. Multiple virus properties contribute to the likelihood that the virus will adapt to efficiently transmit in human populations and the potential consequences of that event, including whether the virus is adapted (or poised to adapt) to efficiently infect and transmit in humans, whether the population has pre-existing immunity to the virus, and viral virulence. As a result, monitoring the virulence of circulating animal influenza viruses is one of the key goals of surveillance. The strategies for monitoring the virulence of surveillance viruses are similar to those for monitoring mammalian adaptation and transmissibility, and GoF approaches that enhance virulence and those that enhance infectivity and transmissibility in representative animal models benefit surveillance through similar mechanisms. Thus, these benefits are discussed collectively in Section 15.3.4.

15.4.5 Benefits of GoF to the Development of Vaccines and Therapeutics

15.4.5.1 Vaccine Development Benefit 1: Development of New Influenza Vaccine Candidates

Standard methods for production of seasonal influenza vaccines have posed challenges for the production of vaccines targeting highly pathogenic avian influenza strains such as H5N1, in part because the wild type HPAI viruses are lethal to embryonated eggs, the main medium used for influenza vaccine production.[1546] In addition, egg-based production systems are not amenable to rapid scale-up due to their reliance on the egg supply, which would pose a major problem if a novel pandemic virus emerged off production cycle. For these reasons, researchers are exploring a variety of other platforms for the production of vaccines for avian influenza viruses with pandemic potential. GoF approaches that enhance virulence benefit the production of one of these platforms, live attenuated influenza vaccines (LAIVs).

15.4.5.1.1 Benefits and Limitations of GoF Approaches

Live attenuated influenza vaccines (LAIVs) are an attractive platform for pandemic vaccines for several reasons: (1) the route of administration mimics the route of natural infection to trigger the generation of mucosal and cell-mediated immunity, which is difficult to generate but is important for achieving robust and long-term protection against mucosal pathogens such as influenza, (2) LAIVs are quicker and cheaper to manufacture than inactivated vaccines due to higher yields per egg and the fact that inactivation and protein purification steps are not required, and (3) LAIVs can be easily administered via intranasal drops or spray.[1547] The major concern associated with LAIVs is their potential to regain virulence in people, through reversion or the acquisition of compensatory mutations.[1548] For that reason, the WHO recommends serial passaging of LAIV candidates during the non-clinical phase of *in vivo* toxicity and safety testing, to determine whether the LAIV is genetically stable or recovers virulence upon passage in

[1546] Baz M *et al* (2013) H5N1 vaccines in humans. *Virus Res* 178: 78-98
[1547] Ibid.
[1548] Ibid.

animals.[1549,1550] In accordance with these recommendations, multiple candidate LAIVs have been subjected to serial passaging in animals.[1551,1552,1553,1554]

15.4.5.1.2 Benefits and Limitations of alt-GoF Approaches

There are no alternative approaches that can provide similar information on the safety of LAIV candidates.

Several alternative vaccine platforms which do not rely on GoF for their development, such as recombinant vaccines, are also being explored. These vaccine platforms have strengths and limitations relative to LAIVs (GoF). For example, adjuvanted, inactivated vaccines may provide broad-spectrum immunity but require multiple doses to confer high levels of protection.[1555]

15.4.5.1.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

A variety of vaccine platforms are being explored for the development of vaccines targeting avian influenza viruses with pandemic potential. LAIVs have several characteristics that are desirable for pandemic vaccines, but a major concern associated with their use is that the LAIV may recover virulence upon growth in people. GoF approaches are uniquely capable of demonstrating whether LAIV strains recover virulence upon growth *in vivo*, a critical aspect of vaccine safety testing prior to the conduct of clinical trials. Other types of vaccines in development have strengths and weaknesses relative to LAIVs. The type or types of vaccines that will ultimately prove to be most effective for avian influenza viruses is not yet clear based on vaccinology research conducted to date. Given the need for effective pandemic influenza vaccines, pursuing all promising strategies for vaccine development in tandem, including LAIVs, will ensure that an effective vaccine is achieved in the shortest possible period of time.

15.4.5.2 Vaccine Development Benefit 2: Targeted Mutagenesis to Remove Virulence Markers from Vaccine Viruses

Most seasonal influenza vaccines are derived from whole vaccine viruses that are produced in eggs.[1556] Existing production systems may also be used for the production of pre-pandemic vaccines and will be used for the production of pandemic vaccines in response to the emergence of a novel pandemic strain. The development of vaccines based on highly pathogenic avian influenza (HPAI) viruses such as H5N1 presents several challenges: (1) wild type viruses must be handled under biosafety level 3 (BSL-3) containment due to their high pathogenicity and (2) wild type viruses are lethal in chick embryos, the medium used for production of most influenza vaccines in the US.[1557] Thus, these viruses must be attenuated in order to be safely and efficiently propagated in eggs for vaccine production. The multibasic cleavage site in the influenza HA protein is a major determinant of viral virulence in eggs and chickens.

1549 WHO Expert Committee on Biological Standardization. (2010) Recommendations to assure the quality, safety and efficacy of influenza vaccines (human, live attenuated) for intranasal administration. *WHO Technical Report Series No 977, 2013*. The World Health Organization,, Geneva, Switzerland pp. 163-196.

1550 The World Health Organization. (2005) WHO guidelines on nonclinical evaluation of vaccines. *WHO Technical Report Series, No 927, 2005*, Geneva, Switzerland, pp. 32-63.

1551 Jang YH, Seong BL (2012) Principles underlying rational design of live attenuated influenza vaccines. *Clinical and experimental vaccine research* 1: 35-49

1552 Han P-F *et al* (2015) H5N1 influenza A virus with K193E and G225E double mutations in haemagglutinin is attenuated and immunogenic in mice. *Journal of General Virology* 96: 2522-2530

1553 Baz M *et al* (2013) H5N1 vaccines in humans. *Virus Res* 178: 78-98

1554 Sedova ES *et al* (2012) Recombinant influenza vaccines. *Acta Naturae* 4: 17-27

1555 Baz M *et al* (2013) H5N1 vaccines in humans. *Virus Res* 178: 78-98

1556 Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.

1557 Suguitan AL *et al* (2006) Live, Attenuated Influenza A H5N1 Candidate Vaccines Provide Broad Cross-Protection in Mice and Ferrets. *PLoS Med* 3: e360

In addition to attenuating HPAI viruses through reassortment with attenuated, high-yield vaccine backbone strains (e.g., PR8), as is standard for the production of all influenza vaccines in eggs, vaccine manufacturers typically remove the HA multibasic cleavage site through targeted mutagenesis to further attenuate the vaccine virus, enabling safe and efficient production of vaccine in eggs (or cells) under BSL-2 conditions. In the future, other conserved determinants of virulence in the HA and NA proteins of avian influenza (AI) viruses could be similarly deleted from AI vaccine viruses in order to further improve the safety of the vaccine production process.

15.4.5.2.1 GoF Approaches – Benefits and Limitations

As discussed above (Section 16.4.2), GoF approaches, in particular forward genetic screens and serial passaging, represent efficient and effective methods for discovering novel viral genetic and phenotypic traits that contribute to virulence. This information provides a foundation for follow-up LoF studies that aim to determine how to *attenuate* virulence, the goal of vaccine virus development, through mutation or deletion of those traits.

15.4.5.2.2 Alt-GoF Approaches – Benefits and Limitations

Several alt-GoF approaches can be used to discover novel virulence factors, including comparative analysis of surveillance data, comparative analysis of the sequences of wild type viruses with varying levels of virulence, use of replication incompetent viruses, and LoF forward genetic screens. As discussed above, each of these approaches has critical limitations for the discovery of novel virulence traits relative to GoF approaches. Namely, comparative sequence analysis and LoF screens are practically limited to the investigation of known virulence traits due to the high genetic diversity among influenza viruses and the inefficiency of screening mutants for attenuated virulence, respectively, and the relevance of novel traits identified using *in vitro* replication-incompetent systems in the context of the complex host environment is unknown.

However, following the identification of novel genetic traits that contribute to virulence, targeted mutagenesis can be used to identify particular mutations within that genetic region that lead to attenuated virulence. This LoF approach can also be used to demonstrate that the attenuating effect of particular mutations is conserved in other virus strains.

15.4.5.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The strengths and limitations of GoF and alt-GoF approaches for the discovery of virulence traits that can be eliminated from vaccine viruses to improve the safety and efficacy of the vaccine production process are summarized in Table 15.24. GoF approaches represent the most efficient and effective strategies for discovery novel genetic traits that contribute to the virulence of influenza viruses. However, GoF approaches cannot be used to identify or confirm genetic changes that are sufficient to *attenuate* the virulence of wild type strains, which is the goal of vaccine virus development. LoF approaches, namely targeted mutagenesis, are uniquely capable of identifying genetic changes (mutations or deletions) that attenuate virulence, as well as demonstrating that the attenuating consequences of those mutations are conserved across multiple virus strains. Taken together, these approaches may enable the development of novel virulence traits that can be mutated to attenuate virulence, which can be applied to the production of AI vaccine viruses to further improve the safety of the vaccine production process.

Table 15.24. Summary of the Benefits of GoF Approaches That Enhance Virulence in Mammals		
Benefits to Vaccine Development – Targeted Mutagenesis to Remove Virulence Markers from Vaccine Viruses		
Approach	**Benefits**	**Limitations**
GoF Experimental Approaches: • Serial passaging of viruses in cells or animals [3] • Genetic modification to introduce genetic traits expected to enhance virulence [1,2,4,5]	• Most efficient and effective strategies for discovering novel virulence traits	• Cannot be used to demonstrate that deletion or mutation of a virulence trait is sufficient to attenuate virulence
Alt-GoF Experimental Approaches: • Genetic modification to introduce traits expected to attenuate virulence (Loss of Function) [4,5,14,19] • Other approaches (see table 15.20)	• Targeted LoF can be used to demonstrate that deletion or mutation of a virulence trait is sufficient to attenuate virulence across multiple virus strains o Goal of application of knowledge to vaccine development	• Approaches are less efficient and effective for the discovery of novel virulence traits than GoF approaches
**Numbers in brackets reference specific experimental approaches in the landscape tables (Supplementary Information).*		

15.4.5.3 Therapeutic Development Benefit 1: Inform the Development of Next-Generation Therapeutics

Only two classes of FDA-approved antivirals are approved for use in the US: the adamantanes, which inhibit the viral M2 protein, and the neuraminidase inhibitors (NAIs), which include zanamivir (Relenza), oseltamivir (Tamiflu), and peramivir (Rapivab).[1558] The adamantanes are no longer recommended for use due to widespread resistance.[1559] Single mutations are sufficient to confer resistance to one or multiple NAIs and have been observed in nature, though NAI-resistance mutations are not yet widespread.[1560] Moreover, the NAIs exhibit limited efficacy, especially if administered more than 48 hours after symptom onset.[1561] Thus, there is an urgent need for the development of new therapeutics against influenza viruses.[1562] Researchers are actively working to develop next-generation influenza therapeutics that directly target viral proteins as well as therapeutics that inhibit host factors that are critical for viral virulence or that exacerbate infection-associated pathology. GoF approaches have potential to benefit the development of both types of therapeutics.

1558 CDC. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Update November 3, 2015. Accessed November 28, 2015.
1559 Ibid.
1560 Ibid.
1561 CDC. Use of Antivirals. Background and Guidance on the Use of Influenza Antiviral Agents. http://www.cdc.gov/flu/professionals/antivirals/antiviral-use-influenza.htm. Last Update February 25, 2015. Accessed November 28, 2015
1562 (2015h) Interviews with influenza researchers.

15.4.5.3.1 Potential Benefits and Limitations of GoF Approaches

As discussed in detail in Section 15.4.3.1, GoF approaches represent the most efficient and effective strategies for discovering novel viral genetic traits that contribute to pathogenicity, which may be good targets for novel therapeutics. In addition, targeted genetic modification of viruses to introduce traits associated with pathogenicity is uniquely capable of demonstrating that particular viral genetic traits are *necessary* and *sufficient* to enhance virulence across multiple virus contexts, which provides a strong mechanistic basis for the role of that viral factor in virulence.

GoF approaches also enable the identification of host factors that are *associated* with virulence and immunopathology, which may be good targets for novel host-targeted therapeutics. However, alt-GoF approaches are needed to confirm the role of a particular host protein in virulence/immunopathology, which provides an important conceptual foundation for the design of therapeutics targeting that protein. Nonetheless, targeted modification to introduce mutations that are expected to enhance pathogenicity (GoF) provides a controlled system for studying the interplay between virus and host factors that contribute to pathogenicity, which is a valuable complement to alt-GoF approaches that perturb the function of host factors, a more blunt approach.

Notably, in both cases, whether inhibiting viral or host factors discovered through GoF approaches is sufficient to attenuate viral replication or infection-associated pathology must be empirically determined using alt-GoF approaches.

15.4.5.3.2 Potential Benefits and Limitations of Alt-GoF Approaches

As discussed in detail in Section 15.4.3.1.3, alt-GoF approaches have significant limitations for the *discovery* of novel viral genetic traits and factors that contribute to virulence. In brief, unless genetically similar viruses are available, approaches that rely on analysis of wild type viruses are limited to the study of traits that are known to be associated with virulence, due to the high genetic diversity among influenza viruses. Comparative analysis of isolates within patients enables the identification of novel adaptive traits that are associated with enhanced virulence over the course of infection, but results may not be broadly conserved in human populations. LoF screens are inefficient and may uncover traits that indirectly contribute to pathogenicity. The use of replication-incompetent viruses enables the identification of novel viral factors that contribute to viral fitness *in vitro*, but the importance of those genetic traits in the context of the complex host environment is difficult to extrapolate. Finally, *in vitro*, virus-free approaches are limited to the study of known virulence traits. However, alt-GoF approaches play a critical role in investigating the function of putative virulence trait, to complement mechanistic information that can be gleaned through GoF approaches. In particular, targeted LoF to confirm that blocking or attenuating the function of a virulence factor attenuates viral replication and/or infection-associated pathology establishes an evidence base for efforts to design therapeutics targeting that virulence factor.

Alt-GoF approaches provide valuable insight into host factors that enhance pathogenicity and contribute to deleterious immune responses. Specifically, the use of targeted knockout animals or pharmacological inhibition of the host factor during infection is uniquely capable of confirming that a host factor contributes to virulence and pathogenicity. These approaches have been extensively used to discover host factors that may be good targets for influenza therapeutics, including inhibitors of the NF-κB signaling pathway, which enhances viral replication through several mechanisms, molecules that suppress levels of

reactive oxygen species, and inhibitors of cytokines/chemokines.[1563,1564,1565,1566,1567] Because inhibition of a host factor is likely to have multi-faceted effects on the immune response during infection, resolving the function of host traits in viral clearance from deleterious immune responses may be difficult using this approach. As a result, other alt-GoF approaches may be used to gain further mechanistic insight into the role of the host factor during infection, including characterization of host immune responses to identify host genes that are up-regulated during infection and LoF targeted genetic modification of viruses to tease apart the role of particular virus-host interactions in pathogenesis.[1568] Because comparative sequence analysis provides minimal mechanistic insight and is of limited utility for discovering novel host factors that contribute to pathogenicity, this alt-GoF approach does not contribute the design of new host-targeted therapeutics.

In addition to designing therapeutics targeting specific virulence factors or pathways (virus or host), several alternative strategies are used to develop novel candidate therapeutics. One alternative approach for designing new therapeutics involves high-throughput screening of small molecule compounds to identify compounds that reduce viral replication *in vitro*, which may identify candidate therapeutics that target viral or host proteins.[1569,1570] This approach has generated promising candidates, including therapeutics that are in Phase III clinical trials in the US.[1571] One drawback of this approach is that it is limited to the identification of compounds that reduce viral replication, which is only one aspect of virulence. Targeting other aspects of virulence, such as viral interactions with the host immune system, may prove to be a more effective therapeutic strategy.

Another alternative approach involves identifying neutralizing monoclonal antibodies (mAbs) targeting virus proteins. These approaches isolating mAbs that bind to particular virus proteins, such as the HA protein, the nucleoprotein (NP), the NA protein, and the M2 protein, from the B cells of convalescent patients or of mice that have been injected with the virus protein of interest.[1572,1573,1574,1575,1576]

1563 Wurzer WJ *et al* (2004) NF-kappaB-dependent induction of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Fas/FasL is crucial for efficient influenza virus propagation. *The Journal of biological chemistry* 279: 30931-30937

1564 Strengert M *et al* (2014) Mucosal reactive oxygen species are required for antiviral response: role of Duox in influenza a virus infection. *Antioxidants & redox signaling* 20: 2695-2709

1565 Zhao D *et al* (2012) Phylogenetic and Pathogenic Analyses of Avian Influenza A H5N1 Viruses Isolated from Poultry in Vietnam. *PloS one* 7: e50959

1566 Kash JC *et al* (2014) Treatment with the reactive oxygen species scavenger EUK-207 reduces lung damage and increases survival during 1918 influenza virus infection in mice. *Free radical biology & medicine* 67: 235-247

1567 McKinstry KK *et al* (2009) IL-10 deficiency unleashes an influenza-specific Th17 response and enhances survival against high-dose challenge. *Journal of immunology* 182: 7353-7363

1568 Cheung CY *et al* (2002) Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 360: 1831-1837

1569 Furuta Y *et al* (2002) In vitro and in vivo activities of anti-influenza virus compound T-705. *Antimicrobial agents and chemotherapy* 46: 977-981

1570 An L *et al* (2014) Screening and identification of inhibitors against influenza A virus from a US drug collection of 1280 drugs. *Antiviral research* 109: 54-63

1571 Toyama Chemical Company, Ltd. Pipeline. https://www.toyama-chemical.co.jp/en/rd/pipeline/index.html. Last Update Accessed November 8, 2015.

1572 Krause JC *et al* (2011a) A broadly neutralizing human monoclonal antibody that recognizes a conserved, novel epitope on the globular head of the influenza H1N1 virus hemagglutinin. *Journal of virology* 85: 10905-10908

1573 Clementi N *et al* (2011) A human monoclonal antibody with neutralizing activity against highly divergent influenza subtypes. *PloS one* 6: e28001

1574 Bodewes R *et al* (2013) In vitro assessment of the immunological significance of a human monoclonal antibody directed to the influenza a virus nucleoprotein. *Clinical and vaccine immunology : CVI* 20: 1333-1337

1575 Shoji Y *et al* (2011) An influenza N1 neuraminidase-specific monoclonal antibody with broad neuraminidase inhibition activity against H5N1 HPAI viruses. *Human vaccines* 7 Suppl: 199-204

1576 Grandea AG, 3rd *et al* (2010) Human antibodies reveal a protective epitope that is highly conserved among human and nonhuman influenza A viruses. *Proceedings of the National Academy of Sciences of the United States of America* 107: 12658-12663

Subsequently, the ability of mAbs to neutralize virus activity is tested. This approach has also generated promising therapeutic candidates, including therapeutics that have entered Phase I clinical trials.[1577,1578] However, mAb-based therapeutics have several drawbacks, including high production costs and the need for injection-based delivery.[1579]

15.4.5.3.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The strengths and limitations of GoF and alt-GoF approaches for the development of new therapeutic candidates are summarized in Table 15.25, below. GoF approaches represent the most efficient and effective strategy for discovering novel viral virulence factors that may be good therapeutic targets, but follow-up alt-GoF approaches are needed to confirm that inhibiting the function of a particular viral factor is sufficient to attenuate or block viral replication and/or infection-associated pathology. Alt-GoF approaches are best-suited for discovering novel host factors that contribute to virulence and immunopathology. However, GoF approaches can be used to gain further mechanistic insight into the function of the host protein during infection, which strengthens the evidence base for developing new therapeutics targeting that host factor. Two completely different approaches for generating new therapeutic candidates are screening libraries of small molecule compounds for their ability to inhibit viral replication *in vitro* and isolating monoclonal antibodies that neutralize essential virus activities by directly binding to virus proteins, both of which have generated promising therapeutic candidates that have entered clinical trials. Given that influenza viruses readily acquire mutations that confer resistance to therapeutics and that different types of therapeutics may be most effective against various influenza subtypes, a wide repertoire of therapeutics is needed to best protect the public against the range of influenza threats that exist in nature. Pursuing all promising pathways for therapeutic development in tandem, including GoF approaches, is the best strategy to achieve this goal.

[1577] HHS funds 2 experimental flu treatments. CIDRAP. http://www.cidrap.umn.edu/news-perspective/2015/09/hhs-funds-2-experimental-flu-treatments. Last Update September 29, 2015. Accessed November 8, 2015.
[1578] Visterra Pipeline. http://www.visterraine.com/pipeline/pipeline.html. Last Update Accessed November 8, 2015.
[1579] HHS funds 2 experimental flu treatments. CIDRAP. http://www.cidrap.umn.edu/news-perspective/2015/09/hhs-funds-2-experimental-flu-treatments. Last Update September 29, 2015. Accessed November 8, 2015.

Table 15.25. Summary of the Benefits of GoF Approaches That Enhance Virulence in Mammals

Benefits to Therapeutic Development – Develop New Candidate Therapeutics		
Approach	**Benefits**	**Limitations**
GoF #1: GoF Experimental Approaches: • Serial passaging of viruses in cells or animals [3] • Genetic modification to introduce genetic traits expected to enhance virulence [1,2,4,5]	• Most efficient and effective strategies for discovering novel viral virulence traits that are conserved across multiple virus strains, which may be good targets for new therapeutics	• Cannot demonstrate that inhibition of a given virulence factor is sufficient to attenuate pathogenesis • Limited utility for the discovery of novel host factors that contribute to virulence, relative to alt-GoF approaches
Alt-GoF #1: Alternative Experimental Approaches: • Genetic modification to introduce traits expected to attenuate virulence (Loss of Function) [4,5,14,19] • Host-focused approaches [10-13, 22] • Other approaches (see Table 15.20)	• Most efficient and effective strategies for discovering novel host factors that contribute to virulence, which may be good targets for new therapeutics • Can be used to demonstrate that blocking or attenuating the function of a viral virulence trait is sufficient to attenuate pathogenesis	• Results in immunocompromised hosts may not translate to healthy populations • Limited utility for the discovery of novel viral factors that contribute to virulence, relative to GoF approaches
Alt-GoF #2: High-throughput screening of small molecule compounds to identify those that inhibit viral replication *in vitro*	• Approach has generated several promising therapeutic candidates	• Limited to the discovery of compounds that inhibit viral replication, which is only one aspect of pathogenesis
Alt-GoF #3: Identify neutralizing monoclonal antibodies (mAbs) targeting particular virus proteins	• Approach has generated several promising therapeutic candidates	• mAb-based therapeutics have several drawbacks, including high production costs and the need for injection-based delivery

**Numbers in brackets reference specific experimental approaches in the landscape tables (Supplementary Information).*

15.4.6 Benefits to Decision-Making in Public Health Policy

Evaluation of the virulence of circulating animal influenza viruses detected through surveillance informs assessment of their pandemic risk, which informs prioritization of investments in pre-pandemic preparedness initiatives, such as pre-pandemic vaccine development. This GoF benefit to decision-making in public health policy is discussed in detail in Section 15.3.5, as evaluation of the transmissibility of animal influenza viruses similarly informs pandemic risk assessments and downstream decision-making.

15.5 Influenza Viruses: Detailed Analysis of the Benefits of GoF Research That Leads to Evasion of Existing Natural or Induced Adaptive Immunity

15.5.1 Overview of the Influenza GoF Landscape

This assessment describes the benefits of GoF experimental approaches that are reasonably anticipated to lead to evasion of existing natural or induced adaptive immunity. In this section, an overview of GoF approaches in this phenotypic category and describe the scientific outcomes and/or products of each approach.

15.5.1.1 Serial Passaging of Viruses in the Presence of Cognate Antibodies

Serial passaging of viruses in the presence of cognate antibodies may lead to the acquisition of mutations that allow the virus to escape neutralization by the antibody. This experiment can be performed in cell culture using monoclonal antibodies, convalescent sera from infected individuals, post-infection ferret sera, or in animals that have been vaccinated or previously exposed to influenza viruses. Sequencing of emergent antibody escape viruses identifies amino acid substitutions that are sufficient to confer antigenic change, which provides a foundation for follow-up studies investigating the molecular basis of antigenic differences between strains. Additionally, sequencing viral isolates at multiple stages of the selection process and determining the effect of amino acid substitutions on viral fitness and other virus phenotypes provides insight into the evolutionary mechanisms driving antigenic drift. Finally, when performed *in vitro* using monoclonal antibodies, the location of escape mutations reveals potential antibody epitope sites.

15.5.1.2 Forward Genetic Screen to Identify Mutations That Alter Antigenicity

Forward genetic screens involve random mutagenesis of the HA protein followed by characterization of the antigenicity of mutants using the hemagglutination inhibition (HAI) assay or other assays, in order to identify amino acid substitutions that do and do not lead to antigenic change. Follow-up studies may determine the consequences of antigenicity-altering mutations on other virus phenotypes, such as viral fitness and pathogenicity. As for serial passaging experiments, the identification of amino acid substitutions that confer antigenic change provides a foundation for studies investigating the molecular basis of antigenic differences. In addition, comprehensive forward genetic screens can be used to define the 'antigenic landscape' of the HA protein– that is, which substitutions the HA protein will tolerate and which of those substitutions cause antigenic drift.

15.5.1.3 Targeted Modification of Viruses to Introduce Mutations That Are Expected to Alter Antigenicity

A final GoF approach that may lead to viruses that evade existing adaptive immunity involves targeted genetic modification to introduce mutations that are expected to alter antigenicity, followed by antigenic characterization of the mutant virus using the HAI assay or other assays. Of note, mutations may be identified through GoF approaches, such as serial passaging of viruses in the presence of cognate antibodies, or alt-GoF approaches, such as comparative analysis of historical sequences. This approach demonstrates that a particular mutation or set of mutations is necessary and sufficient to alter antigenicity, which provides a foundation for follow-up studies investigating the molecular basis of antigenic differences between strains.

Notably, the level of pre-existing immunity to a given wild type influenza virus in the human population varies depending on when the strain circulated in human populations and other factors. For example, only those people born prior to or shortly after the 1968 H3N2 influenza pandemic may possess pre-existing

immunity to the 1968 H3N2 virus today, acquired through exposure to the 1968 strain or antigenically similar descendants by natural infection or vaccination. In contrast, a large fraction of the population is expected to have pre-existing immunity to recently or currently circulating seasonal influenza viruses or to seasonal influenza viruses that have recently served as the basis for vaccine strains. Consequently, the degree to which laboratory-generated strains that evade pre-existing immunity, created using any one of the GoF approaches described above, pose an increased risk to human health at the population level is strain-specific (i.e., depends on the history of that virus strain and the level of existing immunity in the human population).

With this caveat in mind, the scope of the benefit assessment for this GoF phenotype includes seasonal and pandemic influenza viruses. (Pandemic influenza viruses include the 1918 H1N1 pandemic virus, the 1957 H2N2 pandemic virus, and the 1968 H3N2 virus, but not the 2009 H1N1 pandemic (H1N1pdm) virus, which is now circulating seasonally.) Of note, although only a small (elderly) fraction of the population has pre-existing immunity to the 1918 H1N1 pandemic virus through natural exposure to the 1918 strain or its early descendants, vaccination against the 2009 H1N1pdm virus has been shown to afford cross-protection against the 1918 H1N1 virus. Specifically, vaccination of mice or ferrets using the monovalent or trivalent form of the inactivated 2009 H1N1pdm vaccine reduced morbidity and mortality associated with subsequent infection with the 1918 H1N1 pandemic virus.[1580,1581,1582] (For a more detailed description of these data, see the online supplemental material.) These data, coupled with the fact that most neutralizing antibodies elicited by infection with H1N1pdm have been found to be broadly neutralizing (against strains as divergent as H5N1),[1583] strongly suggest that natural infection with the 2009 H1N1pdm virus would also cross-protect against infection with the 1918 H1N1 virus.[1584] However, this phenomenon has not yet been formally investigated. Taken together, this body of research suggests that the US and global populations may have significant pre-existing immunity to the 1918 H1N1 virus, though how and whether such immunity would mitigate the consequences of an outbreak caused by the 1918 virus is uncertain. For this reason, antigenic escape studies utilizing the 1918 H1N1 virus and its early descendants were included in the analysis of the benefits of GoF research that leads to evasion of existing natural or induced immunity. To the authors' knowledge, such studies have not been performed utilizing the reconstructed 1918 H1N1 virus. However, several antigenic escape studies involving a classical swine H1N1 isolate from 1930 (A/Swine/Iowa/15/30), the HA sequence of which more closely resembles the 1918 HA sequence than the sequence of any other existing isolate,[1585] were identified. These studies are included in the landscape tables for the "Evasion of Existing Natural or Induced Immunity" section (Supplemental Information) and their benefits are evaluated here. Of note, this 1930 strain is not known to infect humans, although more recent classical swine influenza viruses can infect people.

In contrast, because human populations do not have widespread immunity to animal influenza viruses (i.e., avian viruses[1586] and swine viruses[1587]), no approaches involving these viruses meet this phenotypic criterion. Therefore, this section does not include studies that investigate the mechanisms underlying

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1580 Easterbrook JD *et al* (2011) Immunization with 1976 swine H1N1- or 2009 pandemic H1N1-inactivated vaccines protects mice from a lethal 1918 influenza infection. *Influenza Other Respir Viruses* 5: 198-205

1581 Medina RA *et al* (2010) Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus. *Nat Commun* 1: 28

1582 Pearce MB *et al* (2012) Seasonal trivalent inactivated influenza vaccine protects against 1918 Spanish influenza virus infection in ferrets. *Journal of virology* 86: 7118-7125

1583 Wrammert J *et al* (2011) Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med* 208: 181-193

1584 Personal communications from influenza researchers (January 2016).

1585 Yu X *et al* (2008) Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 455: 532-536

1586 Jernigan DB, Cox NJ (2015) H7N9: Preparing for the Unexpected in Influenza. *Annual Review of Medicine* 66: 361-371

1587 Skowronski DM *et al* (2012) Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2v). *J Infect Dis* 206: 1852-1861

antigenic drift of avian strains in response to selection pressure from vaccination or the chicken immune system, nor any other studies focused on animal influenza strains. Note that because these studies may lead to the acquisition of mutations in the influenza HA protein, which is a critical determinant of mammalian adaptation, transmissibility, and virulence, these studies may result in the generation of viruses with altered virulence, infectivity, and transmissibility from a "human" perspective. However, whether and what phenotypic changes are likely to arise cannot be anticipated with certainty.

Finally, GoF approaches may also lead to the generation of influenza viruses that are capable of evading recognition by the host innate immune system. Because virus interactions with innate immune factors are critical determinants of virulence, these approaches are evaluated in the "enhanced morbidity and mortality in appropriate animal models" section (15.4).

15.5.2 Overview of the Potential Benefits of GoF Experiments That May Lead to the Generation of Influenza Viruses That Evade Existing Natural or Induced Adaptive Immunity

15.5.2.1 Scientific Knowledge

GoF approaches have the potential to benefit several aspects of scientific knowledge about the antigenic drift of influenza viruses. First, GoF studies that identify mutations that confer antigenic change provide a foundation for follow-up studies investigating the molecular basis of antigenic differences between strains. Second, GoF studies provide insight into the evolutionary mechanisms driving antigenic drift in response to immune pressure. Finally, GoF studies enable the identification of antigenic sites on the HA protein, which can also provide insight into both aspects of antigenic drift described above.

15.5.2.2 Surveillance

GoF approaches that lead to the identification of mutations that alter antigenicity have potential to inform the interpretation of surveillance data for human seasonal influenza by facilitating inference of antigenic phenotype from genotype, in lieu of isolating and antigenically characterizing viruses. Specifically, these data inform the development of models for predicting antigenic phenotype from genotype, or surveillance sequences can be examined for the presence of absence of particular amino acid substitutions that are associated with antigenic change. Either application has the potential to inform the bi-annual selection of strains for the seasonal influenza vaccine.

Because this GoF phenotype is restricted to the study of human seasonal influenza viruses, GoF approaches in this category do not benefit surveillance in wildlife or agricultural animals.

15.5.2.3 Vaccines

GoF approaches have potential to improve the strain selection process for seasonal influenza vaccines in several ways. First, a critical factor in strain selection is analysis of the antigenic characteristics of circulating influenza viruses, to determine whether new antigenic variants have emerged. As described in Section 15.5.2.2, GoF data may facilitate prediction of antigenic phenotype from genotype, which may provide several advantages over the use of traditional, laboratory-based antigenic characterization methods. In addition, GoF approaches have the potential to aid efforts to predict antigenic drift, either directly through the selection and analysis of drifted strains or by informing the development of models for predicting drift. As selected strains sometimes drift during the course of vaccine development, which leads to poor vaccine match, either effort could improve the efficacy of vaccines by enabling deliberate production of "drifted" strains that match circulating strains at the time of vaccine deployment.

15.5.2.4 Therapeutics and Diagnostics

GoF approaches in this phenotypic category are focused on elucidating mechanisms of antigenic drift in response to immune pressure, which is not relevant for the development of therapeutics. (We note that studies that generate escape mutants from candidate monoclonal antibody therapeutics, which are experimentally similar to approaches described above, are discussed in Section 15.7.)

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[1588]

15.5.2.5 Informing policy Decisions

GoF approaches have potential to inform the selection of strains for the seasonal influenza vaccine in several ways, as described in Section 15.5.2.3.

15.5.2.6 Economic Benefits

GoF approaches that inform strain selection for seasonal influenza vaccines may improve the efficacy of seasonal flu vaccines by increasing the likelihood that the vaccine strains will match the strains that are circulating during the target influenza season. Ultimately, this benefit may increase vaccine uptake but otherwise is unlikely to yield economic benefits.

15.5.3 Benefits of GoF to Scientific Knowledge

Influenza viruses circulating in nature acquire mutations in response to immune pressure from human populations that allow the viruses to escape recognition by the adaptive immune system, a process termed "antigenic drift".[1589] As a result, the strain composition of the seasonal influenza vaccine must be updated annually to ensure that the vaccine strains antigenically "match" circulating strains. Research in this area is focused on the influenza HA protein, which is the immunodominant influenza protein and represents the primary component of current influenza vaccines. (The role of other influenza proteins, such as neuraminidase, in the adaptive immune response is not well understood and is an active area of research. Given this uncertainty, this section does not evaluate studies that investigate virus escape from antibodies against non-HA influenza proteins.) The mechanisms underlying antigenic drift of the HA protein and the relationship between genotype and antigenic phenotype are not well understood. One of the knowledge gaps that contributes to this uncertainty is an incomplete understanding of the antigenic sites on the HA protein that are targeted by neutralizing antibodies, as these sites are presumably hotspots for antigenic evolution.[1590] Most work to map antibody epitopes has been conducted using murine antibodies, which exhibit some distinctive antibody binding characteristics relative to human mAbs. Additionally, although the major antigenic sites on the H1 protein were defined in the early 1980s using the lab-adapted A/Puerto Rico/8/1934 (PR8) strain, the antigenic regions of the H1 protein from the 2009 H1N1 pandemic strain

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[1588] New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

[1589] Webster RG *et al* (1982) Molecular mechanisms of variation in influenza viruses. *Nature* 296: 115-121

[1590] O'Donnell CD *et al* (2012) Antibody pressure by a human monoclonal antibody targeting the 2009 pandemic H1N1 virus hemagglutinin drives the emergence of a virus with increased virulence in mice. *MBio* 3

may be different and have been the subject of several preliminary studies.[1591,1592,1593,1594] (Experimentally mapping the antibody epitopes of future pandemic strains will be an important research and public health goal if a new pandemic strain emerges.) Mapping antigenic sites is also important for understanding the molecular basis of neutralizing antibody activity, as well as gaining insight into the mechanisms underlying the cross-protection afforded by broadly neutralizing antibodies (e.g., neutralizing antibodies produced in response to the 2009 H1N1 pandemic virus afford some level of protection against infection with the 1918 H1N1 pandemic virus, which has a related HA sequence, and vice versa).[1595,1596,1597,1598,1599]

In this section, the ability of GoF methods, versus alternative approaches, to address three unanswered questions in this field are evaluated:

- How do influenza viruses evolve antigenically in response to immune pressure? That is, what are the evolutionary mechanisms driving antigenic drift, including the role of different selection pressures (e.g., vaccination) and the interplay between antigenic escape and other virus phenotypes, such as fitness?
- What is the molecular basis of antigenic drift? That is, what amino acid substitutions in the HA protein lead to antigenic change, and what is the biophysical basis of that effect?
- What are the antigenic sites on the HA protein that are targeted by neutralizing antibodies?

For each question in turn, the potential benefits and limitations of relevant GoF approaches and alt-GoF approaches are described, then the benefits of GoF approaches relative to alt-GoF approaches are evaluated. Unique benefits of GoF and alt-GoF approaches are highlighted.

15.5.3.1 Scientific Knowledge Gap 1 – How Do Influenza Viruses Evolve Antigenically in Response to Immune Pressure?

15.5.3.1.1 Potential Benefits and Limitations of GoF Approaches

GoF approaches that involve serial passaging of viruses in the presence of cognate antibodies provide insight into the evolutionary mechanisms driving antigenic drift in response to immune pressure. Both *in vivo* and *in vitro* approaches have unique strengths. Namely, subjecting viruses to selection from the full complement of the animal immune system better mimics the selective pressure viruses experience in humans, while *in vitro* approaches can be conducted using convalescent sera (or isolated antibodies) from people, which may be more relevant to humans than selective pressures in animals. In addition, the *in vivo*

1591 Caton AJ *et al* (1982) The antigenic structure of the influenza virus A/PR/8/34 hemagglutinin (H1 subtype). *Cell* 31: 417-427

1592 O'Donnell CD *et al* (2012) Antibody pressure by a human monoclonal antibody targeting the 2009 pandemic H1N1 virus hemagglutinin drives the emergence of a virus with increased virulence in mice. *MBio* 3

1593 Rudneva I *et al* (2012) Escape mutants of pandemic influenza A/H1N1 2009 virus: variations in antigenic specificity and receptor affinity of the hemagglutinin. *Virus Res* 166: 61-67

1594 Krause JC *et al* (2011b) A Broadly Neutralizing Human Monoclonal Antibody That Recognizes a Conserved, Novel Epitope on the Globular Head of the Influenza H1N1 Virus Hemagglutinin. *J Virol* 85: 10905-10908

1595 Medina RA *et al* (2010) Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus. *Nat Commun* 1: 28

1596 Easterbrook JD *et al* (2011) Immunization with 1976 swine H1N1- or 2009 pandemic H1N1-inactivated vaccines protects mice from a lethal 1918 influenza infection. *Influenza Other Respir Viruses* 5: 198-205

1597 Pearce MB *et al* (2012) Seasonal trivalent inactivated influenza vaccine protects against 1918 Spanish influenza virus infection in ferrets. *Journal of virology* 86: 7118-7125

1598 Manicassamy B *et al* (2010) Protection of mice against lethal challenge with 2009 H1N1 influenza A virus by 1918-like and classical swine H1N1 based vaccines. *PLoS Pathog* 6: e1000745

1599 Wei CJ *et al* (2010) Cross-neutralization of 1918 and 2009 influenza viruses: role of glycans in viral evolution and vaccine design. *Sci Transl Med* 2: 24ra21

approach represents a controlled system for studying the role of selective pressures from prior exposure to influenza viruses through natural infection and/or vaccination in shaping antigenic evolution. In both cases, results from laboratory studies may not translate to the evolution of viruses in human populations in nature and may not be conserved in other virus contexts. Importantly, follow-up studies can determine the effect of antigenic drift on other virus phenotypes, such as fitness, which provides insight into how likely mutations are to persist in a host or in a population once they have arisen.

15.5.3.1.2 Potential Benefits and Limitations of Alt-GoF Approaches

The use of attenuated strains for serial passaging studies, in lieu of wild type strains, represents one type of alt-GoF approach. Two types of attenuated strains are used for serial passaging studies to investigate antigenic evolution mechanisms: the mouse-adapted strain PR8, which is avirulent in people,[1600] and 6:2R strains that contain the HA and NA gene segments from a seasonal strain of interest and the remaining six gene segments from PR8. While use of either type of attenuated strain can provide insight into the basic mechanisms of antigenic evolution, results may not translate to wildtype strains due to differences in disease pathogenesis caused by wildtype versus attenuated strains and other factors. Another potential concern is that relative HA and NA expression levels may be different in the context of a 6:2R, as the effect of HA/NA balance on antigenic drift is as yet unknown. Moreover, 6:2R strains cannot be used to predict the effect of antigenic escape mutations on the fitness of wildtype strains because *in vivo* fitness is a complex, multi-genic trait that is highly dependent on genetic context. As the PR8 strain and 6:2R strains do not efficiently infect ferrets,[1601] these studies are limited to the use of mouse model systems.

Comparative analysis of historical virus sequences that have drifted antigenically over time represents an alternative experimental approach for studying antigenic evolution. Relative to GoF approaches, the strength of the comparative sequence analysis approach is that it provides insight into the antigenic evolution of a wide breadth of influenza viruses in human populations. However, the success of this approach depends on the quality of available surveillance data; some strains have limited numbers of sequences available, and biases in the way that some surveillance data are collected render the data unsuitable or difficult to use. Moreover, given the variability in levels of pre-existing immunity in the population due to differences in infection and vaccination histories, inferring how selective pressures from vaccination and/or prior infection shape antigenic drift may be difficult. An additional limitation is that the historical record is static– that is, it cannot provide insight into mutations that were selected against, which is important knowledge for understanding the pressures and constraints that guide antigenic evolution. Furthermore, the extent of information that can be generated using this approach is constrained by the fact that history has only explored a fraction of the possible antigenic space, for a given influenza subtype. Finally, this approach cannot be used to proactively study the antigenic evolution of currently circulating viruses.

In silico approaches can be also used to investigate mechanisms underlying antigenic drift of influenza viruses. Existing models are largely based on historical sequence data and accompanying antigenic characterization data and have been validated using historical data. As a result, the quality of these models is constrained by the set of limitations described above for the comparative sequence analysis approach. Although models can provide insight into the relationships between genetic and antigenic evolution, their accuracy in predicting future antigenic drift is unknown, thus any predictions must be experimentally validated. Additional experimental data about pathways for antigenic evolution, including data generated using GoF approaches, is needed to improve the quality of existing models.

[1600] Beare AS *et al* (1975) Trials in man with live recombinants made from A/PR/8/34 (H0 N1) and wild H3 N2 influenza viruses. *Lancet* 2: 729-732

[1601] Jin H *et al* (2004) Imparting Temperature Sensitivity and Attenuation in Ferrets to A/Puerto Rico/8/34 Influenza Virus by Transferring the Genetic Signature for Temperature Sensitivity from Cold-Adapted A/Ann Arbor/6/60. *J Virol* 78: 995-998

15.5.3.1.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Table 15.26 summarizes the benefits and limitations of GoF and alt-GoF approaches that provide insight into the antigenic evolution of influenza viruses. Taken together, GoF approaches are uniquely capable of providing in-depth information about the evolutionary mechanisms driving antigenic drift as well as prospective information about the evolution of currently circulating viruses. *In vivo* approaches provide insight into antigenic drift in response to selective pressure from the full complement of the immune system but may not translate to humans, while *in vitro* approaches can provide information about antigenic changes that arise in response to selective pressure from human antibodies but may not translate to complex, *in vivo* scenarios. In either case, lessons learned in the laboratory may not translate to virus behavior in human populations in nature. In contrast, comparative sequence analysis is uniquely capable of providing information about the antigenic evolution of viruses in nature, but is constrained to reactively studying the evolution of historic viruses in limited depth.

Table 15.26. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Immunity		
Scientific Knowledge Benefits – What Are the Evolutionary Mechanisms Underlying Antigenic Drift?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1 [1]*: *In vitro*: Serial passaging of virus in the presence of monoclonal antibodies for one or more passages	• Provide insight into evolutionary mechanisms driving antigenic drift ○ Directly translates to humans, if convalescent sera (or isolated antibodies) from people are used • Provide insight into the consequences of antigenic drift for replicative fitness • Proactive - can be performed using currently circulating viruses	• Artificiality – adaptive changes observed in the laboratory may not be representative of evolution in nature • Narrow breadth – results may not generalize to other virus contexts • Translatability – in vitro results may not translate to humans

GoF #2 [2]:
In vivo: Serial passaging of virus in vaccinated animals or animals with prior exposure to influenza viruses
	• Provide in-depth insight into evolutionary mechanisms driving antigenic drift
	  ○ Selective pressure from full complement of the animal immune system mimics selective pressure in humans
	  ○ Identifies positively and negatively selected traits
	  ○ Controlled system for studying role of selective pressure from prior exposure to influenza viruses through vaccination and/or natural infection
	• Provide insight into the consequences of antigenic drift for other viral phenotypes, such as fitness
	• Proactive – can be performed using currently circulating viruses
		• Artificiality - adaptive changes observed in the laboratory may not be representative of evolution in nature
		• Narrow breadth - results may not generalize to other virus contexts
		• Translatability – results from animal models may not translate to humans

Table 15.26. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Immunity

Scientific Knowledge Benefits – What Are the Evolutionary Mechanisms Underlying Antigenic Drift?		
Experimental Approach	**Benefits**	**Limitations**

Alt-GoF #1 [1]:
Comparative analysis of historical virus sequences
	• Provide insight into evolutionary mechanisms driving antigenic drift
	  ○ Provides information on the natural evolutionary process
	  ○ Directly translates to humans
	  ○ Analyzes broad datasets applicable to many strains
		• Limited by the quality and availability of existing surveillance data
		• Reactive – limited to the study of antigenic evolution that has already occurred in nature
		• Static – cannot identify lost or negatively selected traits
		• Variability in levels of pre-existing immunity in surveillance populations complicate interpretation of selection pressures

Alt-GoF #2 [2]:
In silico, virus free: Use computational or mathematical approaches to build models for prediction of future antigenic drift
	• Gain insight into evolutionary mechanisms of antigenic drift
	• Proactive – can be applied to currently circulating viruses
		• Predictive – does not confirm or correlate phenotypic effects in a biological context
		• Model accuracy – existing models are based on historical data
		  ○ Limited by quality and availability of existing surveillance data
		  ○ Accuracy in predicting future antigenic drift is unknown
		• Cannot predict consequences of antigenic drift on other viral phenotypes

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify approaches described in the landscape tables (Supplemental Information).*

15.5.3.2 Scientific Knowledge Gap 2 – What Is the Molecular Basis of Antigenic Drift?

15.5.3.2.1 Potential Benefits and Limitations of GoF Approaches

Several GoF approaches can be used to discover mutations that lead to antigenic drift, which provides a foundation for follow-up studies investigating the biophysical basis of antigenic change. First, serial passaging of viruses in cells in the presence of cognate sera or monoclonal antibodies, or in animals that have been vaccinated or previously exposed to influenza viruses, leads to the emergence of antigenic escape mutants. Sequencing the HA gene of emergent escape viruses reveals mutations that are sufficient to alter virus antigenicity. This approach is highly efficient and can be applied to any virus, including currently circulating strains. Notably, *in vitro* and *in vivo* selection approaches equally enable the identification of mutations associated with antigenic drift, though the *in vitro* approach is faster and cheaper. Importantly, as multiple mutations may arise during passaging, follow-up studies may be needed to determine which mutation(s) are responsible for the antigenic escape phenotype.

Forward genetic screens, which involve mutagenesis of the HA protein and subsequent characterization of the antigenicity of mutant viruses, represent another GoF approach for identifying mutations that confer antigenic change. Though screening for escape mutants is more labor-intensive than selection methods based on serial passaging, the screening approach is uniquely capable of identifying mutations that do *not* lead to antigenic change, which critically informs efforts to develop models for the sequence-based prediction of antigenicity. In addition, comprehensive mutagenesis of the HA protein enables characterization of the "antigenic landscape" of HA– that is, the set of amino acid substitutions that HA can tolerate and the subset of those that lead to antigenic change. Understanding the antigenic plasticity of the HA protein provides important context for evaluating the molecular basis of antigenic drift and may benefit the development of new influenza vaccines, as described below. Importantly, because of the influence of genetic context on antigenicity, antigenic escape mutations identified through either serial passaging or forward genetic screens may not generalize to other virus strains within the same or different HA subtype.

Finally, targeted genetic modification of viruses to introduce mutations associated with antigenic change, followed by antigenic characterization of mutant viruses, is used to demonstrate that mutations are *necessary* and *sufficient* to alter antigenicity. Notably, these mutations may be identified through GoF approaches, such as serial passaging, or alt-GoF approaches, such as comparative sequence analysis (described below). Subsequently, to determine whether the phenotypic consequences of mutations are functionally generalizable across multiple virus strains, targeted mutagenesis can be used to introduce mutations into new virus strains, followed by antigenic characterization. Together, these results provide a strong foundation for follow-up structural studies to determine the biophysical basis of antigenic differences and critically inform the development of models for the prediction of antigenic phenotype from genotype.

15.5.3.2.2 Potential Benefits and Limitations of Alt-GoF Approaches

Because experiments in this phenotypic category focus on the influenza HA protein, reassortment strains containing the HA and NA genes from a seasonal strain of interest and the remaining six "internal" genes from the lab-adapted, attenuated strain PR8 (6:2R strains) can be used in lieu of wildtype seasonal strains for any of the GoF approaches described above. Due to the fact that a 6:2R strain is attenuated relative to the parental HA/NA donor strain, use of 6:2R strains represents one type of alt-GoF approach. Because the antigenicity of the HA protein is preserved in the context of a 6:2R strain,[1602] 6:2R strains are as

[1602] (2015h) Interviews with influenza researchers.

suitable as wild type strains for the discovery and confirmation of amino acid substitutions that lead to antigenic drift using *in vitro* or mouse model systems. Although 6:2R strains do not efficiently infect ferrets, this limitation does not compromise the utility of 6:2R strains because ferret experiments do not provide unique information about antigenic escape mutations relative to the use of other model systems.

Several alternative experimental approaches can also be used to identify mutations associated with antigenic change. Comparatively analyzing the sequences of natural isolates that have drifted antigenically over time can lead to the identification of mutations that are associated with antigenic change. Even though the major antigenic sites on the HA protein have been mapped, not all mutations within those sites cause antigenic changes and mutations outside those sites may lead to antigenic changes through long-range effects. Current models cannot accurately predict which mutations do or do not lead to antigenic drift, necessitating follow-up experiments to determine which of the identified mutation(s) lead to antigenic drift. The key drawback of this approach is that it is limited to the identification of mutations that have arisen in nature, which represents a fraction of the possible antigenic space.

In silico approaches represent another alt-GoF approach for the identification of mutations associated with antigenic drift. Specifically, computational models based on antigenic, sequence, and HA structural data can be used to predict amino acid substitutions that will alter antigenicity. Although computational approaches can fully explore all possible antigenic configurations, existing models cannot predict mutations that will lead to antigenic change with certainty, thus the phenotypic consequences of any predicted mutation must be confirmed experimentally. Notably, because existing models are primarily based on historical sequence data and accompanying antigenic characterization data, the quality of these models is constrained by the set of limitations described above for the comparative sequence analysis approach. Additional experimental data, including data generated from GoF experiments, is needed for parameterization of improved models.[1603]

Finally, the use of virus-like particles (VLPs) represents a virus-free alternative approach for testing whether particular mutations are *necessary* and *sufficient* to alter antigenicity in lieu of targeted genetic modification of wild type viruses. VLPs used for antigenic drift studies are produced by transfecting mammalian cells with influenza HA and NA expression plasmids.[1604,1605] VLPs containing HA and NA proteins then bud from the cell surface and can be purified from the supernatant and utilized in antigenic characterization assays in place of wild type viruses. Because VLPs do not contain other influenza proteins or influenza genetic material, they are non-infectious. Although the morphology – and, therefore, the antigenicity – of VLPs may differ slightly from that of whole viruses, influenza researchers stated that VLPs generally serve as good approximations for wild type viruses in antigenic characterization assays.[1606]

15.5.3.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Table 15.27 summarizes the benefits and limitations of GoF and alt-GoF approaches that can provide insight into the molecular basis of antigenic drift. Taken together, GoF approaches are uniquely capable of identifying amino acid substitutions that are *necessary* and *sufficient* to alter antigenicity in the context of whole viruses, which provides a critical foundation for follow-up studies to elucidate the biophysical basis of antigenic differences. Furthermore, GoF approaches represent the most efficient and reliable method for uncovering mutations that cause antigenic drift in circulating strains and are uniquely capable

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1603 (2015) Interviews with influenza researchers.
1604 Chen BJ *et al* (2007) Influenza virus hemagglutinin and neuraminidase, but not the matrix protein, are required for assembly and budding of plasmid-derived virus-like particles. *Journal of virology* 81: 7111-7123
1605 Yu X *et al* (2008) Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 455: 532-536
1606 (2015) Interviews with influenza researchers.

of exploring antigenic space to define which mutations do and do not lead to antigenic changes, which can improve predictive modeling efforts. For the purpose of discovering mutations that lead to antigenic change, GoF approaches can be conducted using attenuated 6:2R strains, instead of wild type strains, without compromising the quality and accuracy of the information that is generated. In addition, either 6:2R strains or VLPs can be used in lieu of wild type viruses to confirm that particular amino acid substitutions are necessary and sufficient to confer antigenic change, with the caveat that morphological differences between 6:2R strains or VLPs and their cognate wild type strains may lead to antigenic differences.

Table 15.27. Summary of the Benefits of GoF Approaches That Lead to Evasion of Immunity to Scientific Knowledge

Scientific Knowledge Benefits – What is the Molecular Basis of Antigenic Drift?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1 [1]*: *In vitro*: Serial passaging of virus in the presence of monoclonal antibodies for one or more passages **GoF #2 [2]:** *In vivo*: Serial passaging of virus in vaccinated animals or in animals with prior exposure to influenza viruses	• Discover novel mutations that are sufficient to confer antigenic change o Gain insight into biophysical basis of antigenic differences • Proactive – can be applied to any virus strain, including currently circulating strains	• Associative – information produced may be correlative, not causative • Narrow breadth – results may not generalize to other virus strains
GoF #3 [3]: Forward genetic screen to identify mutations that alter antigenicity	• Discover novel mutations that do and do not cause antigenic drift o Gain insight into biophysical basis of antigenic differences o Gain insight into the antigenic plasticity of the HA protein • Proactive - can be applied to any virus strain, including currently circulating strains	• Narrow breadth - results may not generalize to other virus strains • Screening approach is more labor-intensive than selection approaches
GoF #4 [4,5]: Targeted genetic modification to introduce mutations that are expected to alter antigenicity	• Confirm that mutations are necessary and sufficient to confer antigenic change o Gain insight into biophysical basis of antigenic differences • Proactive - can be applied to any virus strain, including currently circulating strains	• Lack of publication of negative results: Compromises evaluation of whether the function of particular markers is broadly conserved

Table 15.27. Summary of the Benefits of GoF Approaches That Lead to Evasion of Immunity to Scientific Knowledge

Scientific Knowledge Benefits – What is the Molecular Basis of Antigenic Drift?

Experimental Approach	Benefits	Limitations
Alt-GoF #1 [1]: Comparative analysis of historical sequences	• Identify mutations that are associated with altered antigenicity	• Associative – information produced is correlative, not causative • Reactive - limited to the study of antigenic space that has already been explored in nature
Alt-GoF #2 [2]: *In silico*, virus free: Use computational or mathematical approaches to build models for prediction of antigenicity based on genotype	• Predict novel mutations that may lead to antigenic changes • Proactive - can be applied to any virus strain, including currently circulating strains	• Predictive – does not confirm or correlate phenotypic effects in a biological context • Model accuracy – existing models are based on historical data o Historical influenza viruses have explored a fraction of the possible antigenic space o Accuracy in predicting phenotypic consequences of novel mutations is unknown
Alt-GoF #3 [5,6] *In vitro*, virus free: Targeted genetic modification of the HA gene to introduce mutations expected to alter antigenicity using virus-like particles (VLPs)	• Confirm that mutations are necessary and sufficient to confer antigenic change o Gain insight into biophysical basis of antigenic differences • Proactive - can be applied to any virus strain, including currently circulating strains	• Antigenicity of VLP may not mimic that of cognate wild type virus, leading to mis-representative results

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify approaches described in the landscape tables (Supplemental Information).*

15.5.3.3 Scientific Knowledge Gap 3: What Are the Antigenic Sites on the Ha Protein That Are Targeted by Neutralizing Antibodies?

15.5.3.3.1 Potential Benefits and Limitations of GoF Approaches

Serial passaging of viruses in cells in the presence of monoclonal antibodies (mAbs) to select for antibody escape mutants is a classic method for identifying putative antibody binding sites. Specifically, the amino acid positions where mutations arise represent potential antigenic sites, although interpretation of this data is complicated by the fact that mutations outside antibody binding sites can alter the confirmation of HA to impact HA-antibody interactions through long-range effects. In the event that multiple mutations arise within the HA protein, targeted mutagenesis to introduce individual mutations into the parental strain may be used to confirm which mutations are necessary and sufficient to confer escape. Together, these approaches can be used to map the epitope of a particular monoclonal antibody or to comprehensively map antigenic sites through the use of multiple, distinct mAbs. In the latter case, a collection of escape mutants is generated using several different mAbs, and subsequent testing of whether each escape mutant can be neutralized by each mAb (i.e., all possible cross-reactions) reveals conserved and distinct epitopes.[1607,1608,1609] This approach is simple, rapid, and allows for precise mapping of antigenic sites. However, each passaging experiment focuses on the identification of a single antigenic site (i.e., recognized by a particular mAb), such that multiple rounds of passaging with distinct antibodies are required to map multiple antigenic regions.

15.5.3.3.2 Potential Benefits and Limitations of Alt-GoF Approaches

Several alt-GoF approaches can also be used to map the antigenic epitopes of the influenza HA protein. One approach involves the use of cell surface display systems in yeast, bacteria, or bacteriophages. These systems exploit the ability of these organisms to express random peptides or protein fragments from the HA protein on their cell surface. Libraries of mutant bacteria/phages/yeast can then be screened for binding to a monoclonal antibody or post-infection sera, for mapping of the antigenic epitope of a particular antibody or comprehensive mapping of antigenic sites, respectively. The main strength of this approach is that it is high-throughput, allowing for mapping of multiple antigenic sites at once through the use of complex sera or multiple mAbs. However, as the presentation of mapped epitopes may be different in the context of the full virus, experiments with full virus should be performed to validate any findings. (We note that validation would entail determining whether mutagenesis of putative antibody binding sites abrogates antibody neutralization, a GoF experiment.)

Another alternative approach involves analysis of crystal structures of a viral protein (or protein fragment) complexed with a particular mAb. The crystal structure demonstrates precisely where an antibody binds to the HA protein, which can be compared to previous studies to determine whether the epitope is previously known or novel. The main drawback of this approach is that it is labor- and time-intensive and therefore has limited throughput. Additionally, researchers have faced technical limitations, such as difficulty crystallizing full-length HA proteins and radiation damage during the data collection process, which may compromise the quality of the data.[1610]

[1607] Caton AJ *et al* (1982) The antigenic structure of the influenza virus A/PR/8/34 hemagglutinin (H1 subtype). *Cell* 31: 417-427

[1608] Gerhard W *et al* (1981) Antigenic structure of influenza virus haemagglutinin defined by hybridoma antibodies. *Nature* 290: 713-717

[1609] Matsuzaki Y *et al* (2014) Epitope mapping of the hemagglutinin molecule of A/(H1N1)pdm09 influenza virus by using monoclonal antibody escape mutants. *Journal of virology* 88: 12364-12373

[1610] Hong M *et al* (2013) Antibody Recognition of the Pandemic H1N1 Influenza Virus Hemagglutinin Receptor Binding Site. Ibid. 87: 12471-12480

Finally, targeted genetic modification of the HA protein using VLPs, a virus-free approach, can be used to confirm that particular amino acid substitutions are sufficient to confer escape from a particular neutralizing antibody, thereby suggesting that the mutated amino acids lie within the antibody binding site. Although influenza researchers stated that VLPs generally serve as good proxies for their cognate wild type viruses, one concern associated with this approach is that differences in the morphology of the VLP relative to the wild type virus may alter its antigenicity.

15.5.3.3.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Table 15.28 summarizes the benefits and limitations of GoF and alt-GoF approaches that provide insight into the antigenic sites of the HA protein that are targeted by human monoclonal antibodies. Serial passaging of viruses in the presence of antibodies, a GoF approach, represents the only method for mapping the antigenic sites of the HA protein in the context of a full virus. However, the fact that mutations outside of antigenic sites may confer escape through long-range effects complicates interpretation of mutational data from these experiments. In addition, the approach is relatively low-throughput in that each passaging experiment enables identification of a single antigenic site, which is a drawback for experiments that aim to comprehensively map antigenic sites on the HA protein (but not for studies aiming to identify the recognition site of a particular mAb). In contrast, the use of cell surface display systems in yeast, bacteria, or phages represents a high-throughput method for identifying the antigenic sites of particular mAbs or for comprehensively mapping the antigenic sites on a given HA protein. Analysis of the crystal structures of HA-antibody complexes precisely reveals the antibody binding site, but the resources needed and technical challenges associated with this approach render it low-throughput. Confirming the results of an *in vitro* experiment requires determining whether mutating the proposed antigenic sites allows for escape from antibody neutralization, which can be done using whole viruses (GoF) or VLPs (alt-GoF). However, the relevance of all three *in vitro* approaches is limited by the fact that that HA presentation may differ in the context of the full virus.

Table 15.28. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Immunity

Scientific Knowledge Benefits – What are the Antigenic Epitopes on the HA Protein?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1 [1]*: *In vitro*: Serial passaging of virus in the presence of monoclonal antibodies for one or more passages	• Discover putative antigenic epitopes on the HA protein in the context of the full virus	• Associative – if more than one mutation arises during passaging • Mutations outside binding sites may confer antigenic escape through long-range effects • Enables identification of a single antigenic site
GoF #2 [4,5]: Targeted genetic modification to introduce mutations expected to confer antigenic escape	• Confirm putative antigenic epitopes on the HA protein in the context of the full virus	• Mutations outside binding sites may confer antigenic escape through long-range effects • Enables identification of a single antigenic site
Alt-GoF #1 [3]: *In vitro, virus free* Cell surface expression of HA peptides or fragments in yeast/phages/bacteria • Screen library for antibody binding	• Discover putative antigenic epitopes on the HA protein • High-throughput o Enables screening with multiple mAbs or complex sera to map multiple antigenic sites at once	• Simplicity of model system – results may not be recapitulated in the context of the full virus
Alt-GoF #2 [4]: *In vitro, virus free* Analysis of crystal structures of HA-antibody complexes	• Discover antibody binding sites on HA proteins	• Simplicity of model system – results may not be recapitulated in the context of the full virus • Low-throughput – X-ray crystallography is labor-intensive
Alt-GoF #3 [5,6] *In vitro*, virus free: Targeted genetic modification of the HA gene to introduce mutations expected to alter antigenicity using virus-like particles (VLPs)	• Confirm putative antigenic epitopes on the HA protein in the context of a VLP	• Antigenicity of VLP may not mimic that of cognate wild type virus, leading to mis-representative results

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify approaches described in the landscape tables (Supplemental Information).*

15.5.4 GoF Benefits to Surveillance

15.5.4.1 Surveillance Benefit 1: Aid in the Interpretation of Seasonal Influenza Genetic Surveillance Data

The WHO Global Influenza Surveillance and Response System (GISRS) conducts surveillance of seasonal influenza viruses year-round. The major goal of seasonal flu surveillance is to monitor the antigenic evolution of viruses– that is, to detect when new antigenic variants emerge in human populations and to determine their prevalence and geographic distribution.[1611,1612] GISRS is a two-tiered surveillance and public health laboratory system.[1613,1614] A global network of National Influenza Centres (NICs) collect clinical specimens in their countries, perform preliminary analyses such as viral isolation and sub-typing, and forward representative virus isolates to one of six WHO Collaborating Centres (WHOCCs) for further characterization. WHOCCs, which include the CDC and St. Jude Children's Research Hospital in the US, conduct antigenic characterization assays, sequencing, and several other virus characterization assays. These data critically inform WHO-coordinated decisions about which strains to recommend including in the seasonal flu vaccine, which are developed during bi-annual Vaccine Composition Meetings (VCMs).[1615,1616] If surveillance data indicate that a new antigenic variant has emerged and spread geographically, the WHO strain selection committee will recommend updating that component of the vaccine.

Antigenic characterization primarily relies on the hemagglutination inhibition (HAI) assay developed in the 1940s.[1617] Though simple and inexpensive, HAI assays have several significant drawbacks that compromise their utility and reliability for antigenic characterization.[1618,1619] First, viruses may acquire adaptive mutations that alter antigenicity during isolation in eggs or cells, in which case the HAI assay will not report on the true antigenicity of the virus present in the original clinical sample. Second, HAI assays are not standardized and exhibit significant variability in the results obtained by different

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[1611] Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

[1612] Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

[1613] (2015z) Interview with Centers for Disease Control and Prevention representative.

[1614] WHO. Global Influenza Surveillance and Response System (GISRS). http://www.who.int/influenza/gisrs_laboratory/en/. Last Update Accessed December 7, 2015.

[1615] Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

[1616] Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

[1617] Hirst GK (1942) THE QUANTITATIVE DETERMINATION OF INFLUENZA VIRUS AND ANTIBODIES BY MEANS OF RED CELL AGGLUTINATION. *J Exp Med* 75: 49-64

[1618] Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

[1619] Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

laboratories.[1620,1621,1622] Finally, technical issues preclude the use of the HAI assay to characterize the antigenicity of many recent H3N2 viruses. Alternative assays for antigenic characterization are currently used when HAI results are difficult to interpret but are more time-consuming and technically demanding than the HAI assay. Due to the time pressures faced by WHOCCs, particularly in the time period immediately preceding the bi-annual VCMs, neither alternative is a viable replacement for the HAI assay.[1623] Notably, even the length of time needed for shipping samples from NICs to WHOCCs (e.g., two to three months between 2010 and 2012 in the WHOCC London region) precludes consideration of isolates collected close to the VCM dates in strain selection decisions.[1624] These exclusions effectively lengthen the period of time between strain selection and the target flu season, which may adversely affect vaccine match.

15.5.4.1.1 Potential Benefits and Limitations of GoF Approaches

GoF approaches have potential to benefit the surveillance of human seasonal influenza viruses by facilitating the prediction of antigenic phenotype directly from genotype in two ways. First, HA sequences can be inspected for the presence or absence of molecular markers for antigenic drift that were identified through GoF approaches. Second, that same GoF-derived data can be used to improve existing models for predicting antigenicity based on genotype. In either case, that information could supplement phenotypic characterization data, to strengthen the certainty of conclusions about antigenic relationships between strains, or could be used in lieu of phenotypic characterization data. These proximal benefits of GoF to seasonal influenza surveillance may ultimately increase the efficacy of seasonal influenza vaccines by improving strain selection capabilities, discussed further below. In brief, GoF approaches that enable prediction of antigenic phenotype from genotype may improve the quality of the input data used for strain selection decisions by increasing the robustness of antigenic characterization data and, if sequencing is performed on clinical isolates, providing information about the natural antigenicity of strains. Additionally, because sequence data can be collected rapidly and economically and is increasingly being generated at NICs, use of sequence-based approaches for determining antigenicity may increase the quantity of data that can be considered, in particular from the time period immediately prior to VCM meetings. Together, improvements to the quantity and quality of input data upon which strain selection decisions are based will increase the likelihood that recommended strains match those that are circulating during the target flu season, which results in increased vaccine efficacy.

During the current strain selection process, HA sequences are inspected for the presence of amino acid substitutions that are known to be associated with altered antigenicity. Structural modeling may be used to help predict whether the substitution will alter antigenicity in that particular genetic context.[1625,1626] This information can be used to corroborate antigenic characterization data from the HAI assay or can help to resolve antigenicity questions when HAI assay results are difficult to interpret. While this

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1620 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. Ibid. 31: 3209-3221

1621 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

1622 (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

1623 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. *Vaccine* 33: 4368-4382

1624 Ibid.

1625 Schultz-Cherry S *et al* (2014) Influenza Gain-of-Function Experiments: Their Role in Vaccine Virus Recommendation and Pandemic Preparedness. *MBio* 5.

1626 (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

information informs the decision-making process, the utility of these markers is limited by significant uncertainties in the state of this science. First, the ability to reliably predict whether a particular amino acid substitution will confer antigenic change in a new genetic context is poor. Second, because other, as-yet-undiscovered amino acid changes may alter antigenicity, the absence of known markers is not yet meaningful (i.e., does not indicate that the antigenicity of the strain is unchanged).

GoF approaches are critical for addressing both aspects of scientific uncertainty to strengthen the utility of molecular marker data for antigenic change. To strengthen the predictive value of molecular markers for antigenic change, several types of experiments are needed:

- Targeted mutagenesis to introduce known genetic markers for altered antigenicity into new genetic contexts (i.e., validate the antigenic consequences of the marker in a variety of strain contexts), which represents a GoF approach,
- Targeted mutagenesis to determine which amino acid substitutions at a particular site previously associated with antigenic change are sufficient to alter antigenicity, which represents a GoF approach, and
- Experiments that explore the antigenic plasticity of the HA protein, to discover new substitutions that confer antigenic change as well as substitutions that do not alter antigenicity.

To address the third experimental goal, two GoF approaches (serial passaging and forward genetic screens) are capable of uncovering novel mutations that confer antigenic change, and targeted mutagenesis can be used to confirm their causality (also GoF). Although these data will undoubtedly strengthen the predictive value of molecular markers for antigenic change, given the importance of genetic context on influenza biology, significant challenges face any effort to improve the predictive value of such markers to a level that is meaningful. In large part, this barrier derives from the fact that the antigenic plasticity of the HA protein is undefined. If HA can accept a very large number of amino acid substitutions, determining the range of substitutions that do and do not alter antigenicity in a variety of strain contexts is likely to be difficult, if not impossible. If the number of substitutions that HA can accept is limited, then delineating this set of substitutions may be feasible. Influenza researchers felt that results from a limited number of additional GoF experiments, to explore whether known markers are conserved and to define the mutational landscape of antigenic drift, are likely to provide insight into the question of whether this goal is achievable. Finally, the fact that negative results are generally not published in the scientific literature also hinders advancements in this area, as knowing when markers are not conserved critically informs their utility.

GoF data can also be used to improve the quality of computational models for predicting antigenic phenotype from genotype, which represents a different sequence-based approach for predicting antigenicity. Current models cannot accurately predict antigenic phenotype from genotype.[1627] GoF approaches have potential to improve these models in two ways: (1) by generating experimental data about novel antigenic changes that are necessary and sufficient to alter antigenicity, which can be incorporated into datasets used to train the models and (2) by testing predictions of novel mutations that would affect antigenicity that these models make, the results from which will feed back to improve model accuracy. As existing models are primarily trained using historical data (i.e., the sequences and antigenic characterization data from historical isolates), the ability of GoF approaches to explore new antigenic space will complement existing data sources to enhance the predictive capability of these models for currently circulating isolates that are evolving antigenically in new ways. As above, the feasibility of

[1627] (2015h) Interviews with influenza researchers.

developing models that can accurately predict antigenic phenotype from genotype will depend on the antigenic plasticity of the HA protein and other factors, which is currently unknown.

If the landscape of amino acid substitutions that can give rise to antigenic change is large, then molecular markers and computational models may never be robust enough to replace antigenic characterization data generated through laboratory assays. Nonetheless, given the shortcomings of phenotypic assays for characterizing antigenicity, the ability to corroborate laboratory results using sequence-based predictions can significantly strengthen the quality of antigenic characterization data, particularly if clinical specimens are directly sequenced.

15.5.4.1.2 Potential Benefits and Limitations of Alt-GoF Approaches

As described above, GoF approaches have the potential to benefit antigenic surveillance for human seasonal influenza viruses in two ways: (1) by improving the predictive value of molecular markers for antigenic drift and (2) by improving the accuracy of models for predicting antigenic phenotype from genotype. The ability of alternative experimental approaches to similarly strengthen the utility of molecular marker data and predictive models is evaluated to understand whether alt-GoF approaches have the potential to benefit surveillance through either mechanism.

Currently, the predictive value of molecular markers for antigenic drift is limited by three sources of scientific uncertainty: (1) whether markers alter antigenicity in different genetic contexts, (2) whether novel amino acid substitutions at particular sites that are known to be associated with antigenic drift will alter antigenicity, and (3) what other amino acid substitutions confer antigenic change. Characterizing the antigenicity of wild type viruses that contain known molecular markers can demonstrate whether a known marker is associated with altered antigenicity in a new genetic context, but no alt-GoF approaches are capable of validating that the marker is necessary and sufficient to confer antigenic change in a new strain, which is essential for application of that knowledge to surveillance.[1628] Similarly, characterization of wild type viruses is limited to determining whether different mutations at known sites or novel mutations are *associated* with antigenic change. Given the limited accuracy of existing models, predictions of any type must be experimentally confirmed using GoF approaches. Finally, as described in Section 16.5.3.1.3, GoF approaches are uniquely capable of defining the antigenic plasticity of the HA protein, which will determine the feasibility of using molecular marker data to infer antigenic phenotype from genotype at all. However, in all cases, attenuated reassortant strains can be used in lieu of wild type strains because the antigenicity of the 6:2R strain is similar to that of the parental wild type strain.

Existing models for prediction of antigenic phenotype from genotype are largely built and validated using historical data. Though comparative analysis of additional historical sequences may uncover new amino acid substitutions that are associated with antigenic change, such data are unlikely to improve the ability of models to predict the antigenic phenotype of currently circulating viruses, which are evolving in new ways, and also cannot be used to validate those predictions. Thus, unlike GoF approaches, alt-GoF approaches are unable to substantially improve existing models by generating new experimental data about relationships between antigenic phenotype and genotype in a variety of strain contexts. However, several completely different types of data can increase the accuracy of these models and will complement improvements that can be gleaned through the use of GoF data. These additional data sources include crystal structures for the HA proteins from a wider variety of strains as well as data about how various amino acid substitutions affect HA stability, which can be generated using *in vitro*, virus-free approaches.[1629]

[1628] (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

[1629] (2015h) Interviews with influenza researchers.

15.5.4.1.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

GoF approaches that lead to evasion of existing natural or induced immunity have potential to benefit surveillance of human seasonal influenza viruses in two ways: by increasing the utility of molecular markers for antigenic drift and by improving the accuracy of existing models for predicting antigenic phenotype from genotype. Attenuated reassortant strains (i.e., 6:2R strains with PR8) can be used in lieu of wild type strains without diminishing these benefits.

GoF approaches are uniquely capable of discovering new amino acid substitutions that are necessary and sufficient to alter antigenicity as well as determining whether markers are conserved in different strain contexts, which collectively increase the predictive value of molecular markers for antigenic change. Given the importance of genetic context for antigenic phenotype, whether such markers will ever be strongly predictive is unknown. However, GoF approaches to explore the antigenic plasticity of the HA protein are uniquely capable of addressing that question. Alternative experimental approaches cannot provide causative data on molecular markers that contribute to altered antigenicity and are limited to studying antigenic changes that have already occurred in nature, which significantly limits their utility for this application.

GoF approaches are uniquely capable of generating experimental data about novel mutations that are necessary and sufficient to confer antigenic change as well as validating predictions about antigenic phenotype based on the sequences of currently circulating viruses, which will improve the accuracy of existing predictive models that are largely based on historical data. However, alternative types of data, including crystal structures of HA proteins from additional strains, are also needed to improve the quality of existing models and will complement gains achieved through the use of GoF approaches.

Together, molecular markers for antigenic change or predictive models can be used to supplement or replace lab-generated antigenic characterization data used to recommend strains for inclusion in the seasonal influenza vaccine. The strengths and limitations of using molecular markers or predictive models for antigenic evaluation of surveillance isolates, relative to the use of phenotypic assays, are summarized in Table 15.29. Although molecular marker data currently informs strain selection decisions, neither data source is robust enough to replace phenotypic data (and may never be). However, use of these data to supplement phenotypic data has potential to improve the quantity, timeliness, and quality of antigenic characterization data that can be considered during VCM meetings, which will ultimately increase the likelihood that recommended strains match those that are circulating during the target flu season, thereby leading to increased vaccine efficacy. Because molecular marker data are currently used in the strain selection process, new data can be seamlessly incorporated into the existing process, so that the only barrier to realization of this benefit is the need to strengthen the state of the science. Influenza researchers involved in the strain selection process stated that computational modeling could play an important role as well, once existing models are improved.[1630] Notably, realization of all GoF benefits to antigenic surveillance relies on the generation of sequence data directly from clinical samples and at NICs, which enables antigenic evaluation earlier than if viruses are shipped to WHOCCs for laboratory-based antigenic characterization. About one-quarter to one-half of HA sequences were generated at NICs during the 2014 – 2015 flu season (discussed in detail below), and sequencing of clinical samples is carried out and is increasingly common (see Section 15.3.4.2.1), demonstrating that these GoF benefits can be realized immediately.[1631] However, full realization of these benefits necessitates expanding sequencing capabilities at NICs and increasing the number of clinical samples that are directly sequenced.

[1630] (2015e) Influenza Vaccine Strain Selection. Interview with Academic Researcher or Federal Government Representative Involved in the Annual Strain Selection Process for Seasonal Influenza Vaccines.

[1631] (2015n) Personal communication from WHOCC representative.

Table 15.29. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Natural or Induced Immunity

Surveillance Benefits – Aid Evaluation of the Antigenicity of Circulating Seasonal Influenza Viruses

Approach	Benefits	Limitations
GoF #1: Strengthen the predictive value of molecular markers for antigenic change	• Could increase the quality of phenotypic characterization data: o Clinical samples can be directly sequenced o Corroboration of HAI assay results increases the robustness of the data • Could increase the quantity of antigenic characterization data considered during VCM meetings: o As sequencing becomes cheaper and easier, a greater number of viruses may be sequenced than subjected to lab-based antigenic characterization o Because NICs are increasingly capable of sequencing virus samples, sequence-based evaluation enables consideration of isolates collected immediately prior to VCM meetings • Molecular marker data are currently used to interpret seasonal flu surveillance data o New data can be incorporated into the process in the immediate term	• Scientific uncertainties compromise the current utility of molecular markers for antigenic change o Time frame for establishing that knowledge is uncertain, likely to be long-term • Use of molecular markers is inherently predictive • Full realization of benefits depends on expanding sequencing capabilities at NICs
GoF #2: Support development of computational models for predicting antigenicity based on sequence	• Could increase the quality of phenotypic characterization data: o Clinical samples can be directly sequenced o Corroboration of HAI assay results increases the robustness of the data • Could increase the quantity of antigenic characterization data considered during VCM meetings: o As sequencing becomes cheaper and easier, a greater number of viruses may be sequenced than subjected to lab-based antigenic characterization o Because NICs are increasingly capable of sequencing virus samples, sequence-based evaluation enables consideration of isolates collected immediately prior to VCM meetings	• Reliable computational models for predicting antigenicity based on sequence do not yet exist, and their future utility depends on scientific advancements o Timeframe for establishing that knowledge is uncertain, likely to be long-term • Use of computational models is inherently predictive • Full realization of benefits depends on expanding sequencing capabilities at NICs

Table 15.29. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Natural or Induced Immunity		
Surveillance Benefits – Aid Evaluation of the Antigenicity of Circulating Seasonal Influenza Viruses		
Approach	**Benefits**	**Limitations**
Alt-GoF #1: Phenotypic evaluation of antigenicity using the HAI assay or other assays	• Provides a direct readout of the antigenicity of a given virus	• Viruses may acquire adaptive mutations that alter antigenicity during isolation in eggs or cells, rendering results inaccurate • HAI assays are not standardized and exhibit significant variability in the results obtained by different laboratories • Technical issues preclude the use of the HAI assay to characterize the antigenicity of many recent H3N2 viruses • Alternative lab assays for antigenic characterization are time-consuming and more technically demanding • The time needed to ship samples from NICs to WHOCCs for antigenic characterization delays generation of the data o Many isolates are not shipped in time for consideration at VCM meetings

15.5.5 GoF Benefits to the Production of Vaccines

15.5.5.1 Vaccine Development Benefit 1: Improve Strain Selection Capabilities for Seasonal Influenza Vaccines

Antigenic drift of human seasonal influenza viruses necessitates frequent updating of influenza vaccines. Since the early 1970s, the WHO has provided formal recommendations for the strain composition of seasonal influenza vaccines based on year-round influenza surveillance conducted through the GISRS (described above).[1632,1633] Based on analysis of the genetic, antigenic, and epidemiologic characteristics of several thousand influenza isolates collected throughout the year, experts suggest candidate vaccine viruses that are likely to antigenically match the strains that will be circulating during the target flu season.[1634,1635,1636] Because of the long production timescales for influenza vaccines (six to eight months), recommendations must be made nearly one year in advance of the predicted peak of influenza activity for the target season.[1637,1638] Despite the complexity of the data considered and the challenge of predicting dominant strains many months in advance, this process generally works well. Most years, the vaccine is well-matched to circulating strains, capable of preventing influenza-like-illness in approximately 70% of vaccine recipients aged 15 – 64 years.[1639] However, occasionally a rare antigenic variant rises to prominence during the course of vaccine production, as happened during the recent 2014 – 2015 flu season for the H3N2 strain, which results in poor vaccine match and reduced vaccine efficacy.[1640,1641]

Several shortcomings compromise the efficacy of the current strain selection process. First, the timeliness and representativeness of isolates forwarded to WHOCCs by NICs, which form the basis of strain selection recommendations, could be improved. Due to significant lag times between sample collection and shipment (e.g., two to three months between 2010 and 2012 in the WHOCC London region), many isolates cannot be analyzed in time for consideration during VCM meetings, which effectively lengthens the period of time between strain selection and the target flu season. Additionally, the viruses that are forwarded may not be fully representative in terms of geography, climate, age groups, and epidemic timing, due to reductions in the number of hospitals that submit samples to NICs and other funding challenges. Taken together, these shortcomings in existing surveillance networks reduce the quality and

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1632 Oshitani H (2010) Influenza surveillance and control in the Western Pacific Region. *Western Pacific surveillance and response journal : WPSAR* 1: 3-4

1633 WHO. Process of Influenza Vaccine Virus Selection and Development. http://apps.who.int/gb/pip/pdf_files/Fluvaccvirusselection.pdf. Last Update November 19, 2007. Accessed November 22, 2015.

1634 Trivalent influenza vaccines (most common) include one A/H1N1, one A/H3N2, and one B strain. Quadrivalent influenza vaccines include an additional B strain.

1635 Schultz-Cherry S *et al* (2014) Influenza Gain-of-Function Experiments: Their Role in Vaccine Virus Recommendation and Pandemic Preparedness. *MBio* 5

1636 Stöhr K (2013b) Influenza vaccine production. In *Textbook of Influenza*, Frs RGW, Md ASM, Md TJB, ScD RAL (eds), pp 352-370. John Wiley & Sons, Ltd

1637 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

1638 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

1639 Legrand J *et al* (2006) Real-time monitoring of the influenza vaccine field effectiveness. Ibid. 24: 6605-6611

1640 Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. Ibid. 31: 3209-3221

1641 Xie H *et al* (2015) H3N2 Mismatch of 2014-15 Northern Hemisphere Influenza Vaccines and Head-to-head Comparison between Human and Ferret Antisera derived Antigenic Maps. *Sci Rep* 5: 15279

quantity of input data for strain selection decisions, which compromises the accuracy of the process. A second shortcoming of the current strain selection process is its heavy reliance on the HAI assay for antigenic characterization of surveillance isolates, which suffers several significant drawbacks as detailed above in Section 15.5.4.1. A final shortcoming is the inability to reliably predict whether rare antigenic variants will rise to prominence in nature during the vaccine production process, which results in poor vaccine match.

15.5.5.1.1 Benefits and Limitations of GoF Approaches

GoF approaches that lead to evasion of existing natural or induced immunity have potential to address all three shortcomings in the current strain selection process.

First, as discussed in Section 15.5.4.1, GoF approaches have potential to strengthen the predictive value of molecular markers for antigenic drift and to improve the accuracy of existing models for predicting antigenic phenotype from genotype. Either strategy for sequence-based prediction of antigenic phenotype could be used to corroborate lab-generated HAI data in cases where results are difficult to interpret. This supplemental data source could strengthen the robustness of antigenic characterization information, thereby improving the quality of input data for the strain selection decision. Alternatively, sequence-based prediction methods could replace laboratory methods for antigenic characterization. Given that sequence data can be collected rapidly and economically and is increasingly being generated at NIC labs, reliance on sequence data may allow for consideration of a greater number of isolates, including isolates sampled close to the VCM dates. The result, an increase in the quantity of input data for the strain selection decision, would improve the process through a different mechanism. Critically, although molecular marker data informs strain selection decisions, neither molecular marker data nor predictive models are currently robust enough to replace phenotypic data (and may never be). Notably, GoF approaches are uniquely critical for advancing the state of the science for both approaches, although other types of data are also needed to improve predictive models and will complement GoF data on molecular markers of antigenic drift. During the 2014 – 2015 season, an average of 28 – 44% of HA sequences were generated at NICs, depending on the strain (range 0% to 70%), though only 9 – 13% of those sequences were submitted in time for consideration in the February VCM meeting.[1642] Thus, given current diagnostic capabilities at NICs, GoF benefits to sequence-based prediction of antigenicity can be realized in the context of the current surveillance system. However, full realization of this benefit necessitates the expansion of sequencing capabilities at NICs as well as increasing the timeliness of sequencing data generated at NICs.

Second, GoF approaches to experimentally induce drift can be used to predict how circulating viruses may drift in nature, enabling production of vaccines against future, "drifted" strains that will antigenically match circulating viruses at their time of deployment. Specifically, the selection of antibody escape mutants of currently circulating viruses, through serial passaging or forward genetic screens conducted *in vitro* and *in vivo*, enables the identification of HA substitutions that confer escape. Coupled with genetic surveillance data, this information can be used to forecast the antigenicity of the next dominant strain to arise in nature.[1643,1644] However, whether and when such variants will emerge is uncertain, in part because stochastic events in natural evolution may result in the appearance of an unusual mutant that was not selected in the experimental studies. For that reason, this data is not currently incorporated into the strain selection process, and additional research is needed to determine whether it will be useful for predicting

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[1642] (2015n) Personal communication from WHOCC representative.

[1643] Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

[1644] (2015h) Interviews with influenza researchers.

the course and timing of antigenic evolution in the future.[1645] Additionally, researchers emphasized that if this strategy is implemented in the strain selection process, the knowledge base must be regularly updated by performing experiments with currently circulating strains.

Finally, a different approach for predicting antigenic drift involves the use of computational models for antigenic evolution (though computational models could be used in conjunction with experimental data). Existing models for prediction of antigenic drift are built largely using historical data (including paired sequence and antigenic data generated for the purpose of strain selection) and have been validated using historical data.[1646,1647] As a result, their prospective applicability and utility are unknown. Two types of GoF studies are needed to improve the quality of existing models. First, a better understanding of the process of antigenic evolution will provide a foundation for the design of better models. As described above (Section 15.5.3.2), GoF approaches are uniquely capable of providing in-depth information about the evolutionary mechanisms driving antigenic drift as well as prospective information about the evolution of currently circulating viruses, although results may not translate to antigenic evolution of viruses in human populations in nature. Second, influenza modeling experts have stated that developing the ability to predict whether particular amino acid substitutions alter antigenicity in a given genetic context is critical for advancing the quality of these models.[1648,1649] As described in the preceding section, GoF approaches are essential for improving the accuracy of models for prediction of antigenic phenotype from genotype, although other types of data are also needed.

Taken together, utilizing experimental and/or *in silico* approaches to predict whether new antigenic variants are likely to emerge during the course of vaccine production would enable the production of vaccines based on those predicted future strains. This strategy would increase the likelihood that vaccines match the strains that are circulating during their target flu season, which will lead to an overall improvement in vaccine efficacy. One key concern associated with this strategy is that evolutionary predictions are difficult and are unlikely to be correct one hundred percent of the time, even as the science of prediction advances. Importantly, the exact amino acid sequence of the next dominant strain does *not* need to be predicted, but rather its antigenicity (as multiple sequences can fall into the same antigenic "cluster"). In addition, studies have shown that immunization with "antigenically advanced" vaccines, i.e., those that are based on predicted future strains, can protect against currently circulating strains. That is, in addition to stimulating production of new antibodies against the antigenically advanced vaccine strain, vaccination re-stimulates production of old antibodies produced in response to prior vaccines, an effect termed "immunity back-boost."[1650] Thus, even if the prediction is incorrect (i.e., the strain does not drift in nature), pre-emptive vaccination strategies are likely afford some degree of protection.

15.5.5.1.2 Benefits and Limitations of Alt-GoF Approaches for Improving Strain Selection Capabilities

As described above, comparative sequence analysis and *in silico* approaches are capable of identifying new molecular markers that are associated with antigenic change or are predicted to alter antigenicity, respectively. However, that such markers are necessary and sufficient to cause antigenic change across a variety of influenza strains must be confirmed through GoF experiments for these data to be applied to the

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[1645] Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

[1646] Bedford T *et al* (2014) Integrating influenza antigenic dynamics with molecular evolution. *Elife* 3: e01914

[1647] Du X *et al* (2012) Mapping of H3N2 influenza antigenic evolution in China reveals a strategy for vaccine strain recommendation. *Nat Commun* 3: 709

[1648] Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. *Vaccine* 33: 4368-4382

[1649] (2015h) Interviews with influenza researchers.

[1650] Fonville JM *et al* (2014) Antibody landscapes after influenza virus infection or vaccination. *Science* 346: 996-1000

interpretation of genetic surveillance data. Furthermore, alt-GoF approaches are constrained to studying antigenic changes in nature. For these reasons, neither alternative approach provides data that can strengthen the predictive value of molecular markers for antigenic change or improve existing models for predicting antigenic phenotype from genotype.

Comparative sequence analysis can also provide insight into antigenic evolution, which critically complements laboratory evolution studies by generating insights that are directly relevant to the evolution of flu viruses in human populations in nature. However, the ability of comparative sequence analysis to provide mechanistic information about evolution is severely limited relative to GoF approaches. In addition, this alt-GoF approach cannot provide prospective information about the evolution of currently circulating viruses, which is the purpose of using antigenic evolution models to inform the strain selection process. For both reasons, the use of comparative sequence analysis approaches is not sufficient to improve the quality of existing models for antigenic evolution.

In addition to using sequence-based prediction of antigenic phenotype to complement or replace the traditional HAI assay, alternative strategies for improving the antigenic characterization data for surveillance isolates have been pursued. The Consortium for the Standardization of Influenza Seroepidemiology (CONSISE), aims to standardize seroepidemiology for influenza and other respiratory pathogens by developing and publishing consensus laboratory assay protocols, including a protocol for the HAI assay.[1651] Ultimately, these efforts have potential to improve the quality of antigenic data considered during strain selection decisions by ensuring that antigenic data generated at disparate sites are more comparable. A second effort to improve antigenic characterization data involves the development of alternative antigenic characterization assays based on synthetic glycan-coated beads or solid matrices in lieu of the red blood cells that are used for traditional HAI assays. Though these assays have greater potential for standardization and automation than the HAI assay, alternative assays to date have had limited success. Because of the acute time pressure faced by WHOCCs, particularly leading up to VCMs, replacement of the HAI assay with the more time-intensive but also more accurate virus neutralization or micro-neutralization assays is not practical.[1652]

Several alternative approaches have potential to improve the strain selection process through completely different mechanisms. First, increasing the timeliness, representativeness, and availability of surveillance isolates would improve the accuracy of strain selection decisions by augmenting the quality of the input data upon which those decisions are based. Key elements of efforts to strengthen influenza surveillance systems include improving national surveillance systems, public health laboratories, and reporting and virus sharing procedures in developing countries.[1653] To that end, between 2004 and 2014, the CDC invested more than $150 million toward building sustainable lab capacity and NICs and other international laboratories in over 40 less developed countries around the world, such as India, Cambodia, Vietnam, and Egypt. The CDC also works closely with Ministries of Health to ensure that they are conducting epidemiological surveillance, including the collection of "metadata" about patient demographics, whether patients have been treated with antivirals or were vaccinated, and other factors along with clinical samples.[1654] The WHO and other WHO member countries also provide support, in the form of funding, technical expertise, and guidance. However, given that resources for public health are

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[1651] Van Kerkhove MD *et al* (2013) The consortium for the standardization of influenza seroepidemiology (CONSISE): a global partnership to standardize influenza seroepidemiology and develop influenza investigation protocols to inform public health policy. *Influenza Other Respir Viruses* 7: 231-234

[1652] Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. *Vaccine* 33: 4368-4382

[1653] Ibid.

[1654] (2015z) Interview with Centers for Disease Control and Prevention representative.

limited and governments have many competing priorities, sustaining and building upon gains in these areas that have occurred in the wake of the 2009 pandemic will continue to pose a major challenge.[1655,1656]

Other lines of research and new technologies have potential to fundamentally change current influenza virological surveillance strategies and activities and may also lead to improved strain selection. For example, an improved understanding of the spatiotemporal distribution of viruses and the factors that influence the geographic spread of viruses could help target surveillance efforts and may also inform prediction of whether and when antigenic variants detected in a particular region are likely to arise.[1657] Deep sequencing of surveillance isolates and systems biology approaches to analysis of such data may provide insight into the role of host-pathogen interactions in the antigenic evolution of viruses, which could also influence vaccination strategies and the strain selection process.[1658] In these and other cases, because the state of the science and/or technology is preliminary, whether and when these approaches will have a demonstrated impact on strain selection for seasonal influenza vaccines is unknown.

15.5.5.1.3 Benefits and Limitations of Alternative Approaches for Improving the Efficacy of Seasonal Flu Vaccines Through Other Mechanisms

In addition to improving strain selection capabilities, several completely different strategies can be used to increase the efficacy of seasonal flu vaccines. These strategies are described in detail in Section 15.2.4.3.4 and are briefly summarized here. First, a universal or broad-spectrum flu vaccine would obviate the need for yearly production of strain-specific vaccines. However, influenza and vaccinology experts disagree about the scientific feasibility of developing a universal vaccine, and one expert felt that a ten to twenty year time frame for development is optimistic. Second, several scientific and technical advancements could shorten production timelines for strain-specific vaccines, which would enable strain selection closer to the start of flu season, presumably increasing the likelihood that the correct strains will be chosen. New vaccine platforms, such as recombinant vaccines, can be rapidly scaled up and have shorter production timelines than egg- and cell-based vaccines. However, the one recombinant vaccine on the market accounts for less than 1% of total seasonal influenza vaccine produced annually, and although several other virus-free vaccine platforms are in development, the length and expense of licensure processes for new vaccines will delay their widespread availability. In addition, it is unclear at what point virus-free vaccines will make up a large enough market share that strain selection meetings could be shifted back (which would compromise the ability of egg- and cell-based vaccine manufacturers to produce vaccine in time for the start of flu season). Incorporating adjuvants into existing egg- and cell-based vaccines would allow for a smaller quantity of antigen to be used per vaccine dose, thus enabling production of the same number of doses in a shorter period of time. However, no US-licensed seasonal vaccines include adjuvants. Although an active area of research, adjuvanted vaccines must undergo standard FDA licensing procedures for new vaccines and thus are unlikely to be broadly available in the near future. Finally, GoF research that enhances virus production enables the development of higher-yield CVVs, which shortens vaccine production timelines by increasing the rate of bulk antigen production. It should be noted that manufacturers already initiate production of at least one component of the seasonal vaccine "at risk" in advance of the VCM meeting, in order to produce sufficient vaccine by the start of flu season. For that reason, it is not clear whether the ability to shorten production timelines for egg- and cell-based vaccines would trigger a shift in the timing of the VCM or would lead manufacturers to delay

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[1655] Ampofo WK *et al* (2013a) Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 31: 3209-3221

[1656] Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Ibid. 33: 4368-4382

[1657] Ibid.

[1658] Ibid.

initiation of bulk antigen production so that all components are produced after the meeting results are publicized.

15.5.5.1.4 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Taken together, GoF approaches are uniquely capable of strengthening the predictive value of molecular markers for antigenic change and play a critical role in improving models for predicting antigenic phenotype from genotype as well as models for predicting antigenic drift. Although alternative experimental approaches can provide other types of data that also strengthen predictive models, these data complement rather than replace GoF data.

Advancing capabilities in these areas has the potential to benefit the strain selection process for seasonal influenza vaccines in several ways, summarized in Table 15.30. First, using sequence-based prediction of antigenic phenotype to reinforce HAI assay results strengthens the robustness of antigenic characterization data, which provides a foundation for strain selection decisions. Second, given that genetic surveillance data are increasingly available from NICs and other sample collection sites, shifting to sequence-based prediction of antigenic phenotype in lieu of laboratory assays has potential to increase the timeliness and quantity of surveillance data that are considered during VCMs. Third, predicting antigenic drift using models or through experimental GoF approaches would enable the development of antigenically advanced vaccines that are likely to match the circulating strains when vaccines are deployed, thereby increasing vaccine efficacy.

Current experimental and modeling efforts cannot yet predict antigenic phenotype from genotype or the timing and direction of antigenic drift. Whether and when such capabilities will be sufficiently accurate to be incorporated into the strain selection process is unknown and depends both on scientific advancements and inherent features of influenza biology. Namely, the antigenic plasticity of the HA protein is not well-characterized but governs the feasibility of each of these predictive efforts. Notably, GoF efforts are also essential for advancing understanding of the antigenic landscape of HA.

Several alternative approaches have potential to improve the strain selection process through different mechanisms. First, efforts to standardize the HAI assay and to develop variant antigenic characterization assays based on synthetic glycans are ongoing, in order to improve the quality of antigenic characterization data upon which strain selection decisions are based. However, these alternative assays are not yet viable replacements for the HAI assay, and the degree to which increased standardization of the HAI assay will improve data quality is uncertain. Initiatives to strengthen global influenza surveillance systems have potential to improve the timeliness, representativeness, and quantity of surveillance isolates that can be considered at VCM meetings but face considerable funding and political barriers. Finally, new technologies such as deep sequencing have the potential to revolutionize influenza virological surveillance activities and may improve strain selection capabilities through unexpected mechanisms. Each of these alternative approaches either complements GoF approaches or addresses different shortcomings in the strain selection process.

Given the complexities involved in coordinating global influenza surveillance and making strain selection decisions under the time pressures imposed by vaccine production timelines, as well as the significant uncertainties in whether and when both GoF and alt-GoF approaches will yield demonstrable benefits to the process, pursuing both GoF and alt-GoF strategies in tandem will ensure that strain selection capabilities are advanced rapidly and to the greatest extent possible.

Finally, several alternative approaches have potential to improve the efficacy of seasonal influenza vaccines through completely different mechanisms. The strengths and limitations of these approaches relative to strategies for improving strain selection capabilities are summarized in Table 15.13. Universal

vaccines represent the only strategy with potential to fully "solve" the vaccine mismatch problem but are in the early stages of development and represent a long-term solution at best. Several approaches, namely the development of virus-free vaccines, the incorporation of adjuvants into existing vaccines, and the development of higher-yield vaccine viruses through GoF approaches that enhance virus production have potential to shorten production timelines for strain-specific vaccines. This adjustment to manufacturing schedules could enable strain selection closer to the start of flu season, which presumably will increase the likelihood of vaccine match. Importantly, all of these approaches complement efforts to improve strain selection capabilities because each approach addresses different underlying gaps in current scientific and technical capabilities that contribute to vaccine mismatch. Thus, influenza vaccine experts recommend pursuing all of these approaches as part of comprehensive strategy for improving the quality of seasonal influenza vaccines.

Table 15.30. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Natural or Induced Immunity

Vaccine Benefits – Increase the Efficacy of Seasonal Flu Vaccines by Improving Strain Selection Capabilities

Approach	Benefits	Limitations
GoF #1: Improve methods for predicting antigenicity based on sequence data: • Strengthen the predictive value of molecular markers for antigenic change • Improve computational models for sequence-based predictions of antigenicity	• Improves the quality of antigenic characterization data upon which strain selection decisions are based: o Clinical samples can be directly sequenced o Corroboration of HAI assay results increases the robustness of the data • Increases the quantity of antigenic characterization data upon which strain selection decisions are based: o As sequencing becomes cheaper and easier, a greater number of viruses may be sequenced than subjected to lab-based antigenic characterization o As the number of NICs that generate sequence data increases, sequence-based evaluation enables consideration of isolates collected close to VCM dates • Molecular marker data are currently used to interpret seasonal flu surveillance data o New data can be incorporated into the process in the immediate term	• Scientific uncertainties compromise the current utility of molecular markers for antigenic change, and existing models for prediction of antigenicity are not accurate o The time frame for advancing the state of science in both areas is uncertain, and is likely to be long-term • Use of molecular markers or computational models is inherently predictive o Unlikely to ever replace phenotypic data, which limits ability of this approach to increase the quantity of antigenic characterization data considered • Full realization of benefits depends on expanding sequencing capabilities at NICs
GoF #2: Improve methods for predicting antigenic drift • Experimentally induce drift in circulating viruses • Improve computational models for predicting antigenic drift	• Enables production of vaccines based on future, drifted strains, which • will antigenically match circulating viruses at their time of deployment • Immunization with "antigenically advanced" vaccines can protect against currently circulating strains o Will achieve some degree of protection even if predictions are incorrect	• The prospective accuracy of experimental methods and computational models for prediction of drift is uncertain o The time frame for advancing the state of the science in both areas is uncertain, and may be long-term • Neither approach is currently incorporated into the strain selection process

Table 15.30. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Natural or Induced Immunity

Vaccine Benefits – Increase the Efficacy of Seasonal Flu Vaccines by Improving Strain Selection Capabilities

Approach	Benefits	Limitations

Alt-GoF #1:
Improve laboratory-generated antigenic characterization data
• Standardize HAI assay
• Develop alternative assays for antigenic characterization
	• Improve the quality of antigenic characterization data upon which strain selection decisions are based
	  ▪ Standardization of HAI assay or development of alternative, standardized assays
	• Improve the quantity of antigenic characterization data upon which strain selection decisions are based:
	  ○ Development of alternative assays that are higher-throughput than the HAI assay
		• Efforts to develop new antigenic characterization assays have had limited success to date
		• Standardization of the HAI assay is challenging

Alt-GoF #2:
Strengthen global influenza surveillance networks
	• Increase the timeliness, representativeness, and availability of surveillance isolates, which will increase the quality of the antigenic characterization data upon which strain selection decisions are based
	  ○ Improve national surveillance systems, public health laboratories, and reporting and virus sharing procedures in developing countries
		• Resources for public health are limited and governments have many competing priorities
		  ○ Maintaining and expanding current surveillance capabilities is challenging

Alt-GoF #3: Alternative lines of research: • Improved understanding of the spatiotemporal distribution of viruses and factors that influence geographic spread • Deep sequencing of surveillance isolates	• May help target surveillance efforts, thereby increasing the quality of antigenic characterization data upon which strain selection decisions are based • May inform predictions of whether and when antigenic variants detected in particular regions are likely to arise, thereby enabling development of better-matched vaccines • Provide insight into the role of host-pathogen interactions in the antigenic evolution of viruses, which could influence strain selection decisions	• The state of the science in these areas is preliminary ○ Whether, to what extent, and when these approaches will benefit the strain selection process is unknown

15.5.5.2 Vaccine Development Benefit 2: Inform Development of Universal or Broad-Spectrum Flu Vaccines

Because existing influenza vaccines are strain-specific, there is a continued need for production of new influenza vaccines to protect public health. Specifically, seasonal influenza vaccines must be updated annually to accommodate antigenic drift of circulating influenza viruses, and specific vaccines must be produced in response to the emergence of a novel pandemic strain. The long production timescales for current influenza vaccines compromise the quality of seasonal flu vaccines (vis-à-vis the potential for reduced vaccine match) and the availability of flu vaccines during a pandemic (discussed in detail in Section 15.2.4.1). For those reasons, researchers are actively pursuing the development of broad-spectrum flu vaccines, which could protect against multiple strains (a subset of related strains within a subtype, an entire subtype, or multiple subtypes), and "universal" flu vaccines, which could protect against all strains. Either type of vaccine would eliminate the need for an exact match between vaccine strains and circulating seasonal viruses, thus improving the efficacy of seasonal flu vaccines. In addition, universal or broad-spectrum vaccines could be available rapidly during a pandemic or could be used to pre-vaccinate the population against emerging influenza strains, thereby increasing vaccine coverage during a pandemic. However, given the high mutation rate of influenza viruses[1659] and the high immunogenicity of strain-specific regions of the HA protein,[1660,1661] development of a broad-spectrum or universal vaccine represents an extremely challenging prospect.[1662,1663] Scientists are exploring multiple strategies for development of such next-generation influenza vaccines, and both GoF and alt-GoF approaches have potential to inform this process.

15.5.5.2.1 Potential Benefits and Limitations of GoF Approaches

GoF approaches that aim to map the antigenic landscape of the HA protein have potential to inform the development of broad-spectrum and universal influenza vaccines. Specifically, comprehensive forward genetic screens to identify which substitutions the HA protein can tolerate and which of those substitutions alter antigenicity will define the regions of the HA protein could drift (i.e., without significantly compromising the stability of HA and the viability of the virus) as well as how those regions can change antigenically. Defining all possible antigenic configurations of the HA protein provides a foundation for developing a broad-spectrum vaccine (or vaccine cocktail) that protects against a large fraction of the possible antigenic space, thus pre-empting antigenic drift in nature and eliminating the need for annual production of seasonal flu vaccines.[1664] Alternatively, defining those regions of the HA protein that do not mutate may provide a foundation for the development of a "drift-resistant" universal vaccine that targets those regions. Currently, whether either strategy will lead to the development of an effective influenza vaccine is unknown. Given the possibility for compensatory mutations to overcome fitness defects arising from antigenic escape mutations as well as the possibility for multiple mutations to

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1659 Parvin JD *et al* (1986a) Measurement of the mutation rates of animal viruses: influenza A virus and poliovirus type 1. *Journal of virology* 59: 377-383

1660 Gerhard W *et al* (1981) Antigenic structure of influenza virus haemagglutinin defined by hybridoma antibodies. *Nature* 290: 713-717

1661 Caton AJ *et al* (1982) The antigenic structure of the influenza virus A/PR/8/34 hemagglutinin (H1 subtype). *Cell* 31: 417-427

1662 Rudolph W, Ben Yedidia T (2011) A universal influenza vaccine: where are we in the pursuit of this "Holy Grail"? *Human vaccines* 7: 10-11

1663 (2015x) Interviews with Federal Government representative and Influenza researchers with expertise in vaccine development.

1664 (2015w) Interview with Biomedical Advanced Research and Development Authority representative.

contribute to antigenic change,[1665] comprehensive mapping of the antigenic landscape of HA may necessitate evaluation of mutations singly and in combination, either of which represents a labor-intensive project. Additionally, whether findings will be specific to an influenza subtype (or subset of strains within that subtype) or will translate to other influenza subtypes is unknown.

15.5.5.2.2 Potential Benefits and Limitations of Alt-GoF Approaches

Alternative approaches can also provide insight into which regions of HA mutate to alter antigenicity and the spectrum of antigenic configurations the HA protein can assume. First, attenuated reassortant strains (i.e., 6:2R strains with lab-adapted strains such as PR8, comprising the HA and NA genes from a seasonal strain of interest and the remaining six genes from PR8) can be used for forward genetic screens in lieu of wild type strains. As the antigenicity of 6:2R strains is preserved relative to that of the parental seasonal flu strain, these strains are suitable for defining the landscape of antigenic configurations that are possible for the HA protein. However, given epistatic effects, the suite of mutations that HA can "tolerate" may be different in the context of a 6:2R strain versus the wild type strain.

Alternative experimental approaches can also be used to study the antigenic landscape of the HA protein. Comparative analysis of historical isolates can provide insight into mutations that are associated with antigenic drift over time. However, this approach is constrained to studying the fraction of antigenic space that the HA protein has explored in nature. Moreover, this approach cannot provide information about why certain substitutions have not been observed in nature (i.e., mechanisms driving negative selection), which is important context for mapping the spectrum of substitutions that are possible. In addition, the causative effects of mutations identified through comparative sequence analysis must be verified through a GoF experiment. Modeling approaches can, in principle, fully explore antigenic space but cannot yet accurately predict antigenic phenotype from genotype nor the effects of HA mutations on protein stability or viral fitness.

Completely different types of scientific data, generated through alt-GoF approaches, can also inform the development of universal and broad-spectrum influenza vaccines. For example, one method for identifying conserved epitopes involves identifying broadly neutralizing antibodies by characterizing the ability of different monoclonal antibodies to neutralize a variety of strains, followed by antibody epitope mapping.[1666] This knowledge can inform the development of multiple vaccine types. Another method involves prediction of conserved immunogenic regions using *in silico* approaches, which has been used as a basis for the development of peptide-based vaccines.[1667,1668,1669] Some of these vaccine candidates have been shown to be immunogenic in animal studies and Phase I clinical trials.[1670,1671,1672] As all universal vaccines are in early stages of development, whether these approaches will prove to be more or less successful than GoF approaches in stimulating development of a safe, effective, and broad-spectrum influenza vaccine is unknown.

1665 Myers JL *et al* (2013) Compensatory hemagglutinin mutations alter antigenic properties of influenza viruses. *Journal of virology* 87: 11168-11172

1666 Zhu X *et al* (2013b) A unique and conserved neutralization epitope in H5N1 influenza viruses identified by an antibody against the A/Goose/Guangdong/1/96 hemagglutinin. *J Virol* 87: 12619-12635

1667 Gottlieb T, Ben-Yedidia T (2014) Epitope-based approaches to a universal influenza vaccine. *Journal of autoimmunity* 54: 15-20

1668 Stoloff GA, Caparros-Wanderley W (2007) Synthetic multi-epitope peptides identified in silico induce protective immunity against multiple influenza serotypes. *European journal of immunology* 37: 2441-2449

1669 Adar Y *et al* (2009) A universal epitope-based influenza vaccine and its efficacy against H5N1. *Vaccine* 27: 2099-2107

1670 Ibid.

1671 Pleguezuelos O *et al* (2012) Synthetic Influenza vaccine (FLU-v) stimulates cell mediated immunity in a double-blind, randomised, placebo-controlled Phase I trial. Ibid. 30: 4655-4660

1672 Pleguezuelos O *et al* (2015) A Synthetic Influenza Virus Vaccine Induces a Cellular Immune Response That Correlates with Reduction in Symptomatology and Virus Shedding in a Randomized Phase Ib Live-Virus Challenge in Humans. *Clinical and vaccine immunology : CVI* 22: 828-835

15.5.5.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The strengths and limitations of GoF and alt-GoF approaches that inform the development of universal or broad-spectrum influenza vaccines are summarized in Table 15.31. GoF approaches are uniquely capable of defining the antigenic landscape of the influenza HA protein– that is, the spectrum of antigenic configurations that HA can assume and which regions of HA are capable of mutating while preserving virus viability. These data may inform the development of broad-spectrum influenza vaccines, which protect against a large fraction of the possible antigenic space, or universal influenza vaccines, which target regions of the protein that are unable to mutate and thus are drift-resistant. Alternative experimental approaches have significant limitations. Attenuated reassortant strains can be used to explore possible antigenic configurations, but results regarding the fitness consequences of mutations may not translate to wild type strains. Comparative analysis of historical isolates is limited to the fraction of antigenic space that has been explored in nature and cannot provide information on mutations that compromise virus viability. While virus-free approaches can be used to explore new antigenic space, these approaches do not reveal the fitness consequences of mutations either. Finally, existing models cannot accurately predict antigenic phenotype from genotype or predict the fitness consequences of particular mutations.

Mapping the antigenic landscape of the HA protein represents a labor-intensive project, and whether vaccine development strategies based on the information gleaned from this approach will be successful is unknown. Other strategies for developing broad-spectrum and universal vaccines, such as *in silico* prediction of conserved epitopes for the development of peptide-based vaccines, have shown promise. All universal/broad-spectrum vaccine candidates are in early stages of development, and which strategy is likely to be most successful is unknown. Given the challenges for developing universal/broad-spectrum vaccines, pursuing all experimental approaches that support vaccine development in tandem, including GoF approaches, will maximize the likelihood of success, which could have large public health impacts.

Table 15.31. Summary of the Benefits of GoF Approaches That Lead to Evasion of Existing Natural or Induced Immunity

Benefits to Vaccine Development: Inform the Development of Universal Influenza Vaccines

Approach	Benefits	Limitations
GoF #1 [3]*: Comprehensive forward genetic screens to map the antigenic landscape of the HA protein • Identify regions of HA that can drift without compromising virus viability	• Defining all possible antigenic configurations of HA could enable the development of broad-spectrum vaccines that protect against a large fraction of the possible antigenic space • Defining regions of the HA protein that cannot drift could enable the development of "drift-resistant" vaccines targeting those regions	• Whether either strategy will enable the development of more effective influenza vaccines is unknown • Benefits are likely to be long-term o Approach is scientifically challenging and labor-intensive o Whether results will be strain- or sub-type specific is unknown
Alt-GoF #1: Alternative Experimental Approaches for mapping the antigenic landscape of the HA protein • Use of attenuated reassortant strains • Comparative analysis of historical sequences • Computational models for prediction of antigenic phenotype from genotype	• Attenuated reassortant strains and computational models can be used to fully explore antigenic space • Comparative sequence analysis can provide information about substitutions that are associated with antigenic drift over time	• Attenuated reassortant strains cannot provide reliable information about whether and to what extent antigenicity-altering mutations compromise the viability/fitness of wild type viruses • Predictions derived from computational models must be experimentally validated • Comparative sequence analysis is constrained to studying the fraction of antigenic space that nature has already explored • Cannot reveal negatively selected mutations
Alt-GoF #2: Alternative strategies for developing universal flu vaccines. • Experimentally identifying broadly neutralizing antibodies • Prediction of conserved immunogenic regions • Other approaches	• Approach has generated several promising vaccine candidates	• Whether these approaches will lead to the generation of safe and effective vaccines is unknown

**Numbers in brackets reference specific experimental approaches in the landscape tables (Supplementary Information).*

15.6 Influenza Viruses: Detailed Analysis of the Benefits of GoF Research That Leads to Evasion of Vaccines

15.6.1 Overview of Influenza GoF Landscape

This assessment describes the benefits of GoF experimental approaches that are reasonably anticipated to lead to evasion of vaccines in development. In this section, an overview of GoF approaches in this phenotypic category is provided, and the scientific outcomes and/or products of each approach are described.

Serial passaging of a virus in cells in the presence of animal sera produced in response to a vaccine or in vaccinated animals may lead to the emergence of viruses that are resistant to neutralization by vaccine-induced antibodies. This approach is used to test whether and how readily viruses can evolve to evade vaccines in development, for example new vaccine platforms that are more broad-spectrum or resistant to drift than current influenza vaccine platforms, which is an important indicator of the potential field efficacy of the vaccine. Most of these experiments involve next-generation influenza vaccine candidates targeting epitopes other than the globular head domain of the hemagglutinin (HA) protein, the target of current influenza vaccines. Given that the globular head domain of HA is the immunodominant protein of influenza viruses and that these next-generation vaccines are not yet widely available, strains that can overcome the protection afforded by these vaccines are expected to pose a minimal increase in human health risk relative to wild type strains.

Because seasonal influenza vaccines are updated annually, approaches that lead to the generation of vaccine strains that are no longer neutralized by vaccine-induced antibodies are more appropriately described by the "evasion of existing induced immunity" phenotype. In addition, we did not identify any studies involving H5N1 viruses that would be expected to lead to the generation of viruses that cannot be neutralized by the pre-pandemic H5N1 vaccine in the national stockpile.

15.6.2 Overview of the Potential Benefits of GoF Experiments that may Lead to the Generation of Influenza Viruses that are Resistant to Therapeutics

This GoF approach is solely focused on understanding how a virus evolves in response to immune pressure from a vaccine under development. As a result, insights gleaned from this approach do not benefit scientific knowledge, surveillance or policy decisions (because the vaccine has not yet been deployed) or the development of therapeutics and diagnostics.

15.6.2.1 Vaccines

GoF approaches that lead to evasion of vaccines in development benefit the development of new influenza vaccines. Specifically, these approaches demonstrate whether and how readily viruses can drift to escape neutralization by new vaccine candidates, which is an important indicator of their potential field efficacy relative to existing vaccines.

15.6.2.2 Economic Benefits

GoF benefits to the development of new vaccines may have downstream economic benefits. Economic benefits were not explicitly evaluated in this report.

15.6.3 Benefits to Vaccine Development

15.6.3.1.1 Shortcomings in Existing Influenza Vaccines

Because existing influenza vaccines are strain-specific, new seasonal flu vaccines must be produced annually in order to accommodate antigenic drift of circulating influenza viruses, and new pandemic flu vaccines must be produced in response to the emergence of a novel pandemic strain. The production timeline for egg- and cell-based influenza vaccines, which comprise over 99% of seasonal flu vaccine doses produced annually, currently spans six to nine months.[1673] As a result, vaccines are unavailable until many months into a pandemic, and the strains for the seasonal flu vaccine must be chosen six months in advance of the start of the target flu season, which occasionally leads to vaccine mismatch and reduced vaccine efficacy. For these reasons, the influenza research and public health communities are strongly interested in developing a broad-spectrum or universal flu vaccine, which would provide coverage for a wider range of influenza strains (e.g., all seasonal A/H3N2 strains) or would provide coverage of all influenza strains (or all influenza A strains), respectively.[1674,1675] Broad-spectrum or universal flu vaccines would obviate the need for annual production of seasonal flu vaccines and could be used to protect the public in advance of the next influenza pandemic. Multiple researchers and vaccine production companies are actively pursuing the development of broad-spectrum or universal flu vaccines.[1676] Demonstrating whether these vaccine candidates are actually drift-resistant or whether viruses acquire mutations to escape neutralization by candidate vaccines less readily than to existing vaccines is a critical aspect of testing the potential field efficacy of these vaccine candidates.[1677]

15.6.3.1.2 Potential Benefits and Limitations of GoF Approaches

Serial passaging of viruses in cells, in the presence of sera from vaccinated animals, or in vaccinated animals may lead to the emergence of mutant viruses that can no longer be neutralized by vaccine-induced antibodies. Sequencing of emergent escape mutants provides insight into how readily viruses can acquire mutations that confer escape from protective vaccination (i.e., how many mutations are needed to escape neutralization). Follow-up studies characterizing other properties of emergent escape viruses relative to the parental virus, such as fitness, may provide additional insight into how likely vaccine escape mutants are to emerge and persist in human populations. *In vitro* studies provide a proof of principle demonstration of whether viruses can mutate to escape vaccines, but virus behavior in response to relatively simple selection pressures may not translate to human populations. *In vivo* studies involve complex selection pressures that more closely mimic those that a virus will encounter during infection of a vaccinated human host, but results in representative animal models may not translate to human disease.

15.6.3.1.3 Potential Benefits and Limitations of Alt-GoF Approaches

No alternative approaches are capable of evaluating whether viruses can acquire mutations to escape neutralization by candidate vaccines prior to field deployment of the vaccine.

[1673] Stöhr K (2013a) Influenza vaccine production. In *Textbook of Influenza, 2nd Edition*, 2nd Edition edn, pp 352-370.
[1674] Rudolph W, Ben Yedidia T (2011) A universal influenza vaccine: where are we in the pursuit of this "Holy Grail"? *Human vaccines* 7: 10-11
[1675] (2015h) Interviews with influenza researchers.
[1676] Rudolph W, Ben Yedidia T (2011) A universal influenza vaccine: where are we in the pursuit of this "Holy Grail"? *Human vaccines* 7: 10-11
[1677] (2015h) Interviews with influenza researchers.

15.6.3.1.4 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Taken together, GoF approaches are uniquely capable of determining whether and how readily influenza viruses can acquire mutations to escape neutralization by candidate broad-spectrum or universal influenza vaccines, a critical aspect of testing the potential field efficacy of vaccine candidates.

15.7 Influenza Viruses: Detailed Analysis of the Benefits of GoF Research That Leads to Evasion of Therapeutics

15.7.1 Overview of Influenza GoF Landscape

This assessment describes the benefits of GoF experimental approaches that are reasonably anticipated to lead to evasion of therapeutics, including licensed therapeutics and therapeutics in development. In this section, an overview of GoF approaches in this phenotypic category is provided, and the scientific outcomes and/or products of each approach are described.

15.7.1.1 Serial Passaging of Viruses in the Presence of Therapeutics

Serial passaging of viruses in the presence of a therapeutic may lead to the acquisition of mutations that allow the virus to evade inhibition by the therapeutic. This approach is performed to determine whether and how readily a virus evolves resistance in response to selective pressure from a therapeutic and to identify mutations that confer resistance, which provides a foundation for follow-up studies investigating the mechanism of action of the therapeutic and the mechanistic basis of antiviral resistance. When passaging experiments are performed using a new therapeutic candidate with an unknown viral target, this information also helps to identify the therapeutic target, as resistance mutations are most likely to arise in the target protein. Of note, the acquisition of resistance to novel classes of therapeutics is not expected to confer cross-resistance to existing antivirals (i.e., adamantanes or neuraminidase inhibitors). Thus, when these experiments involve drug candidates within new classes of therapeutics, which are not yet widely available, no increase in human health risk is posed by resistant strains. Serial passaging approaches have been performed using cell culture, animal models, and (rarely) human challenge experiments.

15.7.1.2 Forward Genetic Screen to Identify Mutations That Confer Antiviral Resistance

Forward genetic screens involve random mutagenesis of antiviral target proteins (e.g., the influenza neuraminidase protein) followed by screening of mutants to identify those with reduced antiviral susceptibility (e.g., to NAIs). Follow-up studies may determine the consequences of antiviral resistance mutations on other virus phenotypes, such as viral fitness. As for serial passaging experiments, the identification of mutations that confer antiviral resistance provides a foundation for studies to elucidate antiviral resistance mechanisms.

15.7.1.3 Targeted Modification of Viruses to Introduce Mutations That are Expected to Confer Antiviral Resistance

A second approach involves targeted genetic modification of a virus to introduce mutations that are associated with antiviral resistance, which may have been identified through GoF approaches such as serial passaging or through alt-GoF approaches such as comparative analysis of sequences from patients who did and did not respond to antiviral treatment. This experiment serves to demonstrate that a particular mutation or set of mutations is necessary and sufficient to enhance antiviral resistance, which provides a foundation for follow-up studies investigating the mechanistic basis of antiviral resistance.

15.7.2 Overview of the Potential Benefits of GoF Experiments That May Lead to the Generation of Influenza Viruses That Are Resistant to Therapeutics

15.7.2.1 Scientific Knowledge

GoF approaches have potential to benefit scientific knowledge by providing insight into the mechanistic basis of antiviral resistance.

15.7.2.2 Surveillance

GoF approaches that lead to the identification of mutations that confer antiviral resistance have potential to inform the interpretation of influenza surveillance data by facilitating the prediction of antiviral resistance phenotype from genotype, in lieu of isolating and characterizing the antiviral sensitivity of viruses through phenotypic assays. In the context of seasonal flu surveillance, this application has the potential to inform therapeutic recommendations for seasonal flu. In the context of animal flu surveillance, this application has the potential to inform pandemic risk assessments and downstream decision-making about investments in pandemic preparedness initiatives.

15.7.2.3 Policy Decisions

GoF approaches that lead to the identification of molecular markers for antiviral resistance contribute to assessments of the pandemic risk posed by circulating animal influenza viruses, which are based on genetic surveillance data and several other types of data (e.g., epidemiologic data, phenotypic data, etc.). These assessments inform policy decisions related to public health preparedness for novel influenza outbreaks. In particular, data about antiviral resistance can inform decisions about whether to pursue Emergency Use Authorization for new therapeutics in late stages of development, in the event that the strain under assessment is known or predicted to be resistant to existing antivirals.

15.7.2.4 Therapeutics

GoF approaches that lead to evasion of therapeutics have the potential to benefit the development of therapeutics in several ways:

- GoF approaches can be used to screen therapeutic candidates based on how readily various candidates acquire resistance and provide information about whether the emergence of resistance enhances the transmissibility or virulence of resistant viruses, an important aspect of safety testing.
- GoF approaches provide information about the potential field efficacy of the therapeutic and the mechanism of activity of the therapeutic, both of which are critical components of an Investigational New Drug application to the FDA.
- GoF approaches can provide insight into the therapeutic dosing regimens and combination therapies (e.g., cocktails of monoclonal antibodies) that are the least likely to permit evolution of resistance.

15.7.2.5 Vaccines

GoF approaches may benefit the production of vaccines through the identification of conserved markers for neuraminidase inhibitor (NAI) resistance. If present in the parental strain upon which a vaccine strain

is based, these markers can be removed from the vaccine virus through targeted deletion or mutagenesis, which may improve the efficacy and safety of the vaccine production process.

15.7.2.6 Diagnostics

Because the process of developing influenza diagnostics is well-established, GoF research does not inform diagnostic development.[1678]

15.7.2.7 Economic Benefits

GoF benefits to the development of therapeutics may have downstream economic benefits. Economic benefits were not explicitly evaluated in this report.

15.7.3 Benefits to Scientific Knowledge

Two classes of antivirals are FDA-approved for general use in the US: the adamantanes, which inhibit the M2 ion channel,[1679] and the neuraminidase inhibitors (NAIs), which inhibit the activity of the NA protein.[1680,1681] As resistance to adamantanes is widespread in seasonal influenza viruses, only NAIs are recommended for therapeutic use.[1682] Three different NAIs are licensed in the US: oseltamivir (Tamiflu®, FDA-approved in 1999), zanamivir (Relenza®, FDA-approved in 1999), and peramivir (Rapivab®, FDA-approved for emergency use in 2009 and for general use in 2014). Although most circulating strains have been sensitive to all NAIs during recent flu seasons, resistance to oseltamivir was widespread during the 2007 – 2008 and 2008 – 2009 seasons, and resistant strains continue to be sporadically detected.[1683,1684] Strains that are resistant to oseltamivir or zanamivir as well as strains that are resistant to both drugs have been observed in nature, in A/H1N1,[1685] A/H3N2,[1686] and B strains.[1687] Resistance has been linked to a variety of mutations, and in most cases, the mechanisms underlying drug resistance are not well understood in seasonal flu strains or animal flu strains. In addition, the factors that shape whether resistant strains will emerge, spread and persist in human populations, including the contribution of viral factors such as the relative fitness of resistant strains, are unknown.

In this section, the ability of GoF approaches, versus alternative experimental approaches, to address two unanswered questions in this field are addressed:

[1678] New diagnostics for novel influenza viruses are typically real-time PCR assays which include two or three diagnostic targets. The influenza M gene is used as a marker for influenza A, the HA gene is used for sub-typing, and the NA gene may also be included. Developing of a new diagnostic assay simply requires designing new primers and probes for a virus of interest, which requires that the sequences of the M, HA, and NA genes are available.

[1679] Schnell JR, Chou JJ (2008) Structure and mechanism of the M2 proton channel of influenza A virus. *Nature* 451: 591-595

[1680] Kim CU *et al* (1997) Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J Am Chem Soc* 119: 681-690

[1681] Li W *et al* (1998) Identification of GS 4104 as an orally bioavailable prodrug of the influenza virus neuraminidase inhibitor GS 4071. *Antimicrob Agents Chemother* 42: 647-653

[1682] CDC. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Update November 3, 2015. Accessed November 28, 2015.

[1683] Dharan NJ *et al* (2009) Infections with oseltamivir-resistant influenza A(H1N1) virus in the United States. *JAMA* 301: 1034-1041

[1684] Hauge SH *et al* (2009) Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. *Emerg Infect Dis* 15: 155-162

[1685] Gubareva LV *et al* (2001) Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *J Infect Dis* 183: 523-531

[1686] Abed Y *et al* (2006) Impact of neuraminidase mutations conferring influenza resistance to neuraminidase inhibitors in the N1 and N2 genetic backgrounds. *Antiviral therapy* 11: 971-976

[1687] Fujisaki S *et al* (2012) A single E105K mutation far from the active site of influenza B virus neuraminidase contributes to reduced susceptibility to multiple neuraminidase-inhibitor drugs. *Biochem Biophys Res Commun* 429: 51-56

- What are the genetic traits underlying resistance to NAIs, and what is the mechanistic basis of resistance?

- What selection pressures shape whether and how readily antiviral-resistant strains arise and spread in nature?

For each question in turn, the potential benefits and limitations of relevant GoF approaches and alt-GoF approaches are described, then the benefits of GoF approaches relative to alt-GoF approaches are evaluated. Unique benefits of GoF and alt-GoF approaches are highlighted.

15.7.3.1 Scientific Knowledge Gap 1 – What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?

15.7.3.1.1 Benefits and Limitations of GoF Approaches

Serial passaging of viruses in the presence of one or multiple therapeutics may lead to the emergence of viruses that are resistant to inhibition by the therapeutic. Sequencing emergent antiviral-resistant viruses enables the identification of novel mutations that are sufficient to confer resistance. Selection for resistance studies can be carried out *in vitro*, *in vivo*, in animals or through human challenge experiments. (Human challenge experiments are rare and have only been conducted using human seasonal strains.) Notably, *in vitro* and *in vivo* selection approaches equally enable the identification of mutations associated with antiviral resistance, though the *in vitro* approach is faster and cheaper. The *in vitro* approach is highly efficient and can be carried out using any virus strain, including currently circulating strains. Importantly, as multiple mutations may arise during passaging, follow-up studies may be needed to determine which mutation(s) are responsible for the antiviral resistance phenotype.

Forward genetic screens, which involve random mutagenesis of the NA genes from antiviral-sensitive strains followed by screening of mutants to identify those with reduced antiviral susceptibility, represent another GoF approach for discovering novel mutations that confer antiviral resistance. The screening approach is less efficient than the selection approach but may enable the discovery of rare antiviral resistance mutations that might be out-competed during a selection experiment due to fitness defects. Although in principle this approach could be applied to genes other than NA to uncover mutations that confer antiviral resistance through epistatic effects, the relative inefficiency of mutant screens has practically limited this approach to the NA gene. Depending on the mutagenesis strategy used, follow-up studies may be needed to determine which mutation(s) are responsible for the antiviral resistance phenotype. Additionally, for both the serial passaging and forward genetic screen approaches, results may not translate to other strain contexts.

Targeted genetic modification of parental viruses to introduce mutations associated with antiviral resistance, followed by phenotypic characterization of the antiviral sensitivity of mutant viruses, is used to demonstrate that a mutation or set of mutations is necessary and sufficient to confer resistance. Notably, these mutations may be identified through GoF approaches, such as serial passaging, or alt-GoF approaches, such as comparative sequence analysis (described below). Subsequently, to determine whether the phenotypic consequences of mutations are functionally generalizable across multiple virus strains, targeted mutagenesis can be used to introduce mutations into new virus strains, followed by characterization of antiviral sensitivity. Together, these results provide a strong foundation for follow-up biochemical, cell biological, structural, and other studies to determine the mechanistic basis of antiviral resistance.

15.7.3.1.2 Benefits and Limitations of Alt-GoF Approaches

Because experiments in this phenotypic category focus on the influenza NA protein, reassortment strains containing the NA gene or the HA and NA genes from a seasonal strain of interest and the remaining six or seven genes from the lab-adapted, attenuated strain PR8 (7:1R or 6:2R strains) can be used in lieu of wildtype seasonal strains for either of the GoF approaches described above. Because these strains are attenuated relative to the parental strain, this represents one type of alternative approach. Influenza researchers felt that results about whether mutations do or do not confer antiviral resistance in the context of attenuated reassortant strains are generally reliable but cautioned that results may not be recapitulated in the context of the wild type strain.[1688] In particular, antiviral resistance mechanisms arising from reduced NA expression, which has been documented for oseltamivir resistance,[1689] or from changes to the balance of HA and NA proteins expressed on the surface of the virion may function differently in attenuated reassortant strains. Additionally, 6:2R and 7:1R strains cannot be used to discover or explore antiviral resistance that arises due to mutations in other virus proteins.

Several alternative experimental approaches can also be used to identify mutations that lead to antiviral resistance. Comparative sequence analysis of wild type strains that are antiviral-resistant and antiviral-sensitive enables identification of mutations that are associated with antiviral resistance. However, because of the high genetic diversity among influenza viruses, identifying relevant mutations may be difficult. One notable exception is comparative analysis of patient isolates over the course of antiviral treatment, which is more readily able to identify mutations associated with antiviral resistance due to the genetic similarity among patient isolates. This approach is most commonly used in immunocompromised patients due to their longer course of illness.[1690,1691,1692,1693] While this approach has successfully identified mutations associated with oseltamivir and zanamivir resistance, the ability to opportunistically sample and analyze patient isolates is likely to be relatively rare. In both cases, a causal link between mutations and antiviral resistance must be established through targeted genetic modification. Re-introduction of mutations into the parental viruses (GoF) can be used to demonstrate that mutations are necessary and sufficient to confer antiviral resistance, while deletion of individual mutations from resistant viruses (LoF) can be used to determine which mutations are necessary for antiviral resistance.

Forward genetic screens to identify mutations that restore antiviral sensitivity to antiviral-resistant strains (LoF) represents another alternative approach for discovering genetic traits linked to antiviral resistance. Because this approach involves screening mutants, it is less efficient than GoF approaches for the discovery of antiviral resistance traits, which rely on selection. Additionally, this approach is limited to the study of antiviral-resistant strains that have arisen in nature and cannot be used to proactively identify novel genetic traits that are associated with antiviral resistance. Targeted genetic modification of antiviral-resistant strains to introduce mutations expected to restore antiviral sensitivity can be used to demonstrate that a particular trait is necessary for antiviral resistance. Given that single mutations are typically sufficient to confer resistance to NAIs, targeted LoF and GoF approaches are equally capable of establishing a causal link between a particular genetic trait and antiviral-resistance. However, use of the targeted LoF method relies on the existence of an antiviral-resistant strain carrying a particular resistance

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1688 (2015h) Interviews with influenza researchers.

1689 Bloom JD *et al* (2010) Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. *Science (New York, NY)* 328: 1272-1275

1690 L'Huillier AG *et al* (2015) E119D Neuraminidase Mutation Conferring Pan-Resistance to Neuraminidase Inhibitors in an A(H1N1)pdm09 Isolate From a Stem-Cell Transplant Recipient. *J Infect Dis*

1691 Baz M *et al* (2006) Characterization of Multidrug-Resistant Influenza A/H3N2 Viruses Shed during 1 Year by an Immunocompromised Child. *Clin Infect Dis* 43: 1555-1561

1692 Gubareva LV *et al* (1998) Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. *J Infect Dis* 178: 1257-1262

1693 Kiso M *et al* (2004) Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet* 364: 759-765

mutation of interest in nature, thus LoF is of limited utility for demonstrating that a resistance trait is conserved across multiple strain contexts than its GoF counterpart.

The use of *in vitro*, virus-free systems represents another alternative approach for the study of genetic traits underlying antiviral resistance. Several *in vitro*, virus free systems for the study of NA1 resistance have been used, which rely on ectopic expression of the influenza NA gene in cell culture.[1694,1695] Using these systems, forward genetic screens, which involve random mutagenesis of antiviral-sensitive NA genes of interest followed by ectopic expression of NA mutant libraries and screening for antiviral resistance, can be used to discover novel mutations that confer resistance. Targeted mutagenesis of wild type NA genes can then be used to demonstrate that a particular mutation or set of mutations is necessary and sufficient to confer resistance, as well as to determine whether the phenotypic consequences of the mutation(s) are conserved across multiple genetic contexts. This approach can be successfully used to study mutations that confer resistance by altering the function of the NA protein. However, this approach cannot be used to uncover or to study mutations that confer resistance by altering the expression levels of the NA protein, as has been documented for the H274Y mutation (N1 numbering),[1696] or mutations in other genes that give rise to resistance through epistatic effects. Additionally, given that antiviral-resistance is a continuum, results may not be recapitulated (or be clinically relevant) in the context of the full virus.

Finally, computational models have been used to predict mutations that disrupt binding between NAIs and the NA protein, which are expected to lead to antiviral resistance. While these models can be used to generate hypotheses about antiviral resistance mutations in any virus strain, all predictions must be experimentally confirmed through targeted mutagenesis, a GoF approach. Additionally, this method cannot be used to predict mutations that give rise to resistance through other mechanisms.

15.7.3.1.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Table 15.32 summarizes the benefits and limitations of GoF and alt-GoF approaches that provide insight into the mechanisms underlying viral resistance to NAIs. Taken together, GoF approaches are uniquely capable of identifying mutations that are *necessary* and *sufficient* to confer antiviral resistance across multiple strain contexts, which provides a strong foundation for follow-up studies to elucidate the mechanisms underlying antiviral resistance. GoF approaches also represent the most efficient and effective approach for discovering novel mutations that confer antiviral resistance in any virus strain, as conducting experiments with wild type viruses allows for discovery of the full spectrum of mutations that may confer resistance, including mutations that alter the function or expression level of the NA gene as well as mutations in other virus proteins that cause resistance through epistatic effects. Attenuated reassortant strains may be used in lieu of wild type strains for many of these experiments, but results may not be recapitulated in the context of the wild type viruses, particularly if antiviral resistance arises through mechanisms other than changes to the function of the NA protein.

Alternative approaches can provide valuable insight into the study of antiviral resistance mechanisms but have limitations relative to GoF approaches. Discovering new genetic traits associated with antiviral resistance through comparative analysis of wild type sequences may be difficult. The comparison of patient isolates over the course of antiviral treatment is a notable exception, but opportunities for such studies are likely to be relatively rare. LoF approaches are relatively inefficient for the discovery of novel

[1694] Nivitchanyong T *et al* (2011) Enhanced expression of secretable influenza virus neuraminidase in suspension mammalian cells by influenza virus nonstructural protein 1. *Journal of virological methods* 178: 44-51

[1695] Schmidt PM *et al* (2011) A Generic System for the Expression and Purification of Soluble and Stable Influenza Neuraminidase. *PLoS ONE* 6: e16284

[1696] Bloom JD *et al* (2010) Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. *Science (New York, NY)* 328: 1272-1275

genetic traits associated with antiviral resistance but can be used to demonstrate that a particular mutation is necessary for antiviral resistance. Notably, the targeted LoF approach is often as capable of establishing a causal link between a particular mutation and antiviral resistance as the targeted GoF approach because NAI resistance is often conferred by single mutations; however, the ability of targeted LoF to demonstrate that particular markers are conserved across strain contexts is limited by the number of antiviral resistant strains in nature. *In vitro* virus-free systems can be used to discover and validate mutations in the NA gene that give rise to resistance but are not suitable for the study of resistance mechanisms that involve alterations to gene expression levels or epistatic effects, and results may not be recapitulated in the context of the full virus. Computational models may be used to predict novel mutations that confer resistance by disrupting binding between the NAI molecule and the NA protein, but all predictions must be experimentally confirmed using GoF approaches.

Table 15.32: Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Scientific Knowledge Benefits – What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1 [1]*: *In vitro* approach: serial passaging of antiviral-sensitive virus in cells in the presence of antiviral	• Identify novel mutations that are sufficient to confer antiviral resistance phenotype • Approach is highly efficient • Proactive – can be carried out using any virus strain, including strains that have not yet gain*ed* resistance in nature	• Associative – Information produced is correlative, not causative • Narrow breadth – Results may not generalize to other virus strains
GoF #2 [2]: *In vivo* approach: Serial passaging of antiviral-sensitive virus in animals in the presence of antiviral	• Identify novel mutations that are sufficient to confer antiviral resistance phenotype • Proactive – can be carried out using any virus strain, including strains that have not yet gain*ed* resistance in nature	
GoF #3 [3]: "Passaging in humans" – human challenge experiments • Challenge human volunteers with drug-sensitive influenza strains and treat with antivirals • Compare virus sequences from isolates collected over the course of antiviral treatment	• Identify mutations that are associated with antiviral resistance in vivo • Proactive – can be carried out using any virus strain, including strains that have not yet gain*ed* resistance in nature	• Associative – Information produced is correlative, not causative • Ethical considerations limit the number of experiments that can be carried out • Narrow breadth – Results may not generalize to other virus strains

Table 15.32: Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Scientific Knowledge Benefits – What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?		
Experimental Approach	**Benefits**	**Limitations**
GoF #4 [4]: Forward genetic screen to identify mutations that confer antiviral resistance	• Identify novel mutations that are necessary and sufficient to confer antiviral resistance phenotype • Ability to identify rare mutations that may be out-competed during selection experiments • Proactive – can be carried out using any virus strain, including strains that have not yet gained resistance in nature	• Narrow breadth - Results may not generalize to other virus strains • Forward genetic screen to identify novel antiviral resistance markers is inefficient relative to GoF approaches; practically limited to investigating mutations in the NA protein
GoF #5 [5, 6]: Targeted genetic modification of antiviral-sensitive virus to introduce mutation(s) associated with antiviral resistance.	• Identify mutations that are necessary and sufficient to confer antiviral resistance phenotype • Gain insight into mechanisms underlying antiviral resistance • Proactive – can be carried out using any virus strain, including strains that have not yet gain resistance in nature • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Lack of publication of negative results – Compromises evaluation of whether the function of particular markers is broadly conserved
Alt-GoF #1 [1]: "Passaging in humans" – comparative sequence analysis of patient isolates from multiple time points over the course of antiviral treatment	• Identify mutations that are associated with evolution of antiviral resistance in vivo	• Associative – Information produced is correlative, not causative • Narrow breadth – Results may not generalize to other virus strains • Limited patient availability constrains the number of studies that can be done • Limited to studying strains that infect study subjects

Table 15.32: Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Scientific Knowledge Benefits – What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?

Experimental Approach	Benefits	Limitations
Alt-GoF #2 [2]: Characterization of wildtype viruses Comparative sequence analysis of natural antiviral-resistant and antiviral-sensitive virus strains	• Identify mutations that are associated with antiviral resistance	• Associative – Information produced is correlative, not causative • Limited by the quality and availability of surveillance data • High genetic diversity impairs identification of relevant mutations • Reactive - Limited to study antiviral resistant strains that have already arisen in nature
Alt-GoF #3 (Loss of Function) [3, 8, 9]: • Forward genetic screen to identify mutations that decrease antiviral resistance • Targeted mutagenesis of antiviral-resistant strains to introduce mutations expected to confer antiviral sensitivity	• Identify novel mutations that are necessary for antiviral resistance • Gain insight into mechanisms underlying antiviral resistance	• Forward genetic screen to identify novel antiviral resistance markers is inefficient relative to GoF approaches; practically limited to investigating mutations in the NA protein • Narrow breadth - Results may not generalize to other virus strains • Reactive – Limited to studying antiviral resistant strains that have already arisen in nature • Limited utility for demonstrating functional generalizability of particular markers across multiple strain contexts
Alt-GoF #5 [5, 6, 10]: Use of *in vitro*, virus free systems: • Forward genetic screen to identify mutations that increase antiviral resistance • Targeted genetic modification of antiviral-sensitive NA gene to introduce mutations expected to confer antiviral resistance	• Identify mutations that are necessary and sufficient to confer antiviral resistance • Enables testing of markers in different strain contexts to assess generalizability of previous findings	• Limited to studying resistance mutations/mechanisms that involve altering the function of the NA protein • Simplicity of model system: Results may not be recapitulated in the context of the full virus

Table 15.32: Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics		
Scientific Knowledge Benefits – What are the Genetic Traits Underlying Resistance to NAIs, and What is the Mechanistic Basis of Resistance?		
Experimental Approach	**Benefits**	**Limitations**
Alt-GoF #6 [7]: *In silico*: computer modeling to predict mutations that disrupt binding between NAIs and the NA protein	• Predict mutations that are necessary and sufficient to disrupt antiviral binding to its target protein, which are expected to confer resistance	• Predictive – Does not confirm or correlate phenotypic effects in a biological context • Model accuracy – Utility of the approach depends on the quality of existing models • Limited to studying resistance mechanisms that involve disruption of NAI-NA interaction
**GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify approaches described in the landscape tables (Supplemental Information).*		

15.7.3.2 Scientific Knowledge Gap 2 – What Selection Pressures Shape Whether and How Readily Antiviral-Resistant Strains Arise and Spread in Nature?

15.7.3.2.1 Benefits and Limitations of GoF Approaches

Serial passaging of viruses in the presence of one or more therapeutics to select for antiviral-resistant strains provides insight into whether, how readily, and how antiviral resistance arises in response to selective pressure from therapeutics. These experiments have been conducted *in vitro* and *in vivo*, through animal experiments and human challenge experiments. Due to the simple selection pressures encountered by viruses during passage in cell culture, the *in vitro* approach is less useful than the *in vivo* approach for understanding how selection pressures in humans are likely to drive the emergence of antiviral-resistant viruses. The ability to gain direct insight into emergence of resistance in humans through human challenge experiments is valuable, but ethical considerations severely constrain the number and scope of experiments that can be carried out. Additionally, variability in host factors (e.g., past exposure to influenza viruses) may complicate interpretation of findings. Animal experiments provide a controlled system for studying the emergence of resistance under complex selection pressures, including identifying resistance mutations that arise but are negatively selected within or between hosts. However, results may not translate to human populations.

Additionally, characterizing the fitness, infectivity, and transmissibility of antiviral-resistant viruses generated through GoF approaches, including serial passaging and targeted mutagenesis, may provide insight into how likely resistant viruses are to emerge, spread, and persist in human populations. In particular, the targeted mutagenesis approach provides a controlled system for studying the interplay between antiviral resistance and other virus properties by enabling comparison of genetically similar viruses that differ only (or primarily) in their antiviral sensitivity.

15.7.3.2.2 Benefits and Limitations of Alt-GoF Approaches

Several alternative approaches can be used to gain insight into selection pressures that shape the evolution and spread of antiviral resistance. Comparative analysis of the sequences and phenotypic characteristics of patient isolates over the course of antiviral treatment has potential to provide direct insight into the mechanisms driving emergence of antiviral resistance in people, including identifying resistance mutations that are negatively selected. However, as these studies are typically conducted in immunocompromised patients due to their longer course of illness, results may not be representative of the general population. In addition, relative to animal passaging experiments (GoF), opportunities to conduct studies involving patients are likely to be relatively rare due to ethical considerations.

Comparative analysis of the phenotypic properties (e.g., fitness) of antiviral-resistant and antiviral-sensitive wild type strains can reveal genetic and phenotypic changes that are *associated* with the acquisition of antiviral resistance (including associations between antiviral resistance and other virus properties), which may provide insight into the viral properties that shape the evolution and spread of antiviral resistance in nature. However, this approach has significant limitations. First, the surveillance record is static and cannot provide insight into negatively selected traits. Second, current surveillance efforts, which largely involve consensus sequencing, are unlikely to capture the emergence of rare antiviral-resistant variants. Finally, due to the high genetic diversity among influenza viruses, this approach cannot establish a causal link between the acquisition of antiviral resistance and other phenotypic changes (e.g., changes in viral fitness). For these reasons, comparative analysis of wild type viruses provides limited insight into the evolutionary mechanisms shaping the evolution and spread of antiviral resistance in nature.

Similar to GoF targeted mutagenesis approaches, targeted LoF approaches (i.e., targeted genetic modification of antiviral-resistant strains to introduce mutations that restore antiviral susceptibility) provides a controlled system for studying the interplay between antiviral resistance and other virus phenotypes, such as fitness.

Other alternative approaches are not suitable for the study of evolutionary pressures that shape the emergence and spread of antiviral resistance. *In vitro*, virus free approaches cannot provide insight into how antiviral resistance affects other virus phenotypes, and current computational models cannot account for epistatic effects (e.g., how antiviral resistance affects fitness). The use of attenuated reassortant strains for GoF selection approaches, in lieu of wild type viruses, is of limited utility for studying the evolution of antiviral resistance because the fitness of attenuated strains is altered relative to the wild type strains.

15.7.3.2.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Table 15.33 summarizes the benefits and limitations of GoF and alt-GoF approaches that provide insight into the evolutionary mechanisms underlying acquisition and spread of viral resistance to NAIs. Taken together, GoF approaches, namely serial passaging of viruses in animals in the presence of therapeutics, represent the most efficient and effective strategy for gaining in-depth insight into the viral and host selection pressures that shape the emergence and spread of antiviral resistance. Notably, attenuated reassortant strains cannot be used for these studies because the phenotypic properties that are likely to shape the likelihood that antiviral resistant strains will spread and persist in human populations, such as fitness, are altered in these strains. While gaining direct insight into the behavior of the virus in humans through human challenge studies (GoF) is valuable, these studies are rare due to ethical considerations and interpretation of findings is complicated by variability in host factors, such as past exposure to influenza. Comparative analysis of patient isolates over the course of antiviral treatment can also provide in-depth insight into the evolution of antiviral resistance in people, but studies are typically conducted in immunocompromised patients and thus may not translate to healthy populations. Comparative analysis of wild type isolates provides limited mechanistic insight into the viral or host factors that shape evolution of antiviral resistance. Finally, neither virus-free approaches nor *in silico* approaches can be used to study the interplay between antiviral resistance and other virus phenotypes.

Table 15.33: Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Scientific Knowledge Benefits – What Selection Pressures Shape Whether and How Readily Antiviral-Resistant Strains Arise and Spread in Nature?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1 [1]*: *In vitro* approach: serial passaging of antiviral-sensitive virus in cells in the presence of antiviral	• Provide in-depth insight into whether and how readily antiviral resistance arises, and the underlying evolutionary mechanisms • Proactive – can be carried out using any virus strain, including strains that have not yet gain resistance in nature • Gain associative insight into the interplay between antiviral resistance and other virus phenotypes, such as fitness	• Translatability – Results in cell culture systems may not translate to human populations • Simplicity of selection pressures in vitro render this approach less useful than the in vivo approach • Associative – Does not establish a causal link between antiviral resistance and other virus phenotypes
GoF #2 [2]: *In vivo* approach: Serial passaging of antiviral-sensitive virus in animals in the presence of antiviral	• Provide in-depth insight into whether and how readily antiviral resistance arises, and the underlying evolutionary mechanisms • Proactive – can be carried out using any virus strain, including strains that have not yet gain resistance in nature • Gain associative insight into the interplay between antiviral resistance and other virus phenotypes, such as fitness	• Translatability – Results in animal models may not translate to human populations • Associative – Does not establish a causal link between antiviral resistance and other virus phenotypes
GoF #3 [3]: "Passaging in humans" – human challenge experiments • Challenge human volunteers with drug-sensitive influenza strains and treat with antivirals • Compare virus sequences from isolates collected over the course of antiviral treatment	• Provide in-depth insight into whether and how readily antiviral resistance arises in humans, and the underlying evolutionary mechanisms • Proactive – can be carried out using any virus strain, including strains that have not yet gain resistance in nature • Gain associative insight into the interplay between antiviral resistance and other virus phenotypes, such as fitness	• Ethical considerations limit the number of experiments that can be carried out • Variability in host factors may complicate interpretation of findings • Associative – Does not establish a causal link between antiviral resistance and other virus phenotypes

Table 15.33: Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics		
Scientific Knowledge Benefits – What Selection Pressures Shape Whether and How Readily Antiviral-Resistant Strains Arise and Spread in Nature?		
Experimental Approach	**Benefits**	**Limitations**
GoF #4 [5, 6]: Targeted genetic modification of antiviral-sensitive virus to introduce mutation(s) associated with antiviral resistance.	• Gain insight into the interplay between antiviral resistance and other virus phenotypes, such as fitness • Controlled system – enables comparison of genetically similar viruses that differ only in their antiviral sensitivity phenotype • Proactive – can be carried out using any virus strain, including strains that have not yet gain resistance in nature	• Translatability – Results in cell culture or animal models may not translate to human populations
Alt-GoF #1 [1]: "Passaging in humans" – comparative sequence analysis of patient isolates from multiple time points over the course of antiviral treatment	• Provide in-depth insight into whether and how readily antiviral resistance arises in humans, and the underlying evolutionary mechanisms • Gain insight into the interplay between antiviral resistance and other virus phenotypes, such as fitness	• Limited patient availability constrains the number of studies that can be done • Results from studies involving immunocompromised patients (common) may not be representative of the general population • Limited to studying strains that infect study subjects • Associative – Does not establish a causal link between antiviral resistance and other virus phenotypes

Table 15.33: Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Scientific Knowledge Benefits – What Selection Pressures Shape Whether and How Readily Antiviral-Resistant Strains Arise and Spread in Nature?		
Experimental Approach	**Benefits**	**Limitations**
Alt-GoF #2 [2]: Characterization of wildtype viruses Comparative sequence analysis of natural antiviral-resistant and antiviral-sensitive virus strains	• Gain associative insight into the interplay between antiviral resistance and other virus phenotypes, such as fitness	• Static – Cannot identify lost or negatively selected traits • Limited by the quality and availability of surveillance data • Consensus sequencing is unlikely to capture the emergence of rare antiviral-resistant strains • Associative – Does not establish a causal link between antiviral resistance and other virus phenotypes • Reactive – Limited to studying antiviral resistant strains that have already arisen in nature
Alt-GoF #3 [3, 8, 9]: Targeted mutagenesis of antiviral-resistant strains to introduce mutations expected to confer antiviral sensitivity (Loss of Function)	• Gain insight into the interplay between antiviral resistance and other virus phenotypes, such as fitness • Controlled system – enables comparison of genetically similar viruses that differ only in their antiviral sensitivity phenotype • Identify novel mutations that are necessary for antiviral resistance • Gain insight into mechanisms underlying antiviral resistance	• Translatability – Results in cell culture or animal models may not translate to human populations • Reactive – Limited to studying antiviral resistant strains that have already arisen in nature

** GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets specify approaches described in the landscape tables (Supplemental Information).*

15.7.4 GoF Benefits to Surveillance

Two classes of antivirals are FDA-approved for general use in the US: the adamantanes, which inhibit the M2 ion channel,[1697] and the neuraminidase inhibitors (NAIs), which inhibit the activity of the NA protein.[1698,1699] As resistance to adamantanes is widespread in seasonal influenza viruses, this class of drug is no longer recommended for therapeutic use. Through influenza surveillance, public health professionals monitor the appearance and prevalence of NAI-resistant strains of seasonal influenza viruses circulating in global populations and of animal influenza viruses that have caused human infections.[1700] Data on the prevalence of antiviral-resistant seasonal strains informs therapeutic recommendations developed by the CDC (i.e., which of the three FDA-approved NAIs should be recommended as a first-line treatment).[1701] In the context of surveillance for zoonotic influenza infections in humans, this data informs decision-making about pandemic preparedness initiatives because antiviral resistance is one of the risk elements considered in a pandemic risk assessment.

Resistance to NAIs can be assessed in two ways: through laboratory testing of NAI resistance using the fluorometric 20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid (MUNANA) assay or other assays and/or by inspecting sequences for the presence of mutations that are known to confer NAI resistance. GoF approaches can improve the practice of using molecular markers by enabling the discovery of new antiviral resistance markers and by validating known markers in new strain contexts. This section first reviews these GoF benefits relative to alternative experimental approaches that may provide similar information. Subsequently, the utility of laboratory assays versus sequence-based prediction for characterizing the antiviral sensitivity of surveillance isolates is analyzed. The public health actions that are taken downstream of this assessment are described in Section 16.7.5, below.

15.7.4.1 Using Molecular Markers for Antiviral Resistance to Interpret Surveillance Data

15.7.4.1.1 Current Utility and Shortcomings of Using Molecular Marker Data to Predict the Antiviral Sensitivity Phenotype of Viruses Detected Through Surveillance

Many mutations have been identified that confer resistance to one or multiple NAIs. In part because NAI resistance can arise from one or two mutations, the predictive value of these markers is generally much stronger than that of markers associated with adaptation, transmissibility, and virulence, which are the result of a constellation of genetic changes.[1702,1703,1704] Multiple markers for NAI resistance have been shown to be functionally generalizable, conferring resistance in multiple strain contexts.[1705] In the experience of influenza researchers and government officials involved in surveillance, the presence of a

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1697 Schnell JR, Chou JJ (2008) Structure and mechanism of the M2 proton channel of influenza A virus. *Nature* 451: 591-595

1698 Kim CU *et al* (1997) Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J Am Chem Soc* 119: 681-690

1699 Li W *et al* (1998) Identification of GS 4104 as an orally bioavailable prodrug of the influenza virus neuraminidase inhibitor GS 4071. *Antimicrob Agents Chemother* 42: 647-653

1700 CDC. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Update November 3, 2015. Accessed November 28, 2015.

1701 Ibid.

1702 (2015h) Interviews with influenza researchers.

1703 Sleeman K *et al* (2013) R292K substitution and drug susceptibility of influenza A(H7N9) viruses. *Emerging infectious diseases* 19: 1521-1524

1704 Boivin G (2013) Detection and management of antiviral resistance for influenza viruses. *Influenza and Other Respiratory Viruses* 7: 18-23

1705 Ibid.

validated antiviral resistance marker is strongly predictive for antiviral resistance, though all agreed that sequence-based predictions must be experimentally confirmed. However, the absence of a known resistance marker is not necessarily predictive of antiviral sensitivity, as it is likely that additional mutations or sets of mutations can lead to resistance, in particular to multi-drug resistance. This lack of knowledge about the mutational landscape that permits evolution of antiviral resistance limits the utility of sequence-based approaches for predicting resistance. Moreover, validating known markers in additional strain contexts will further strengthen their predictive value. As discussed in detail in Section 16.7.3.1 above, both GoF and alt-GoF approaches can provide insight into these scientific questions. The relevant findings are summarized here.

15.7.4.1.2 Summary - Benefits of GoF Approaches Relative to Alt-GoF Approaches for Improving the Utility of Molecular Markers for Antiviral Resistance

GoF approaches represent the most efficient and effective strategy for discovering novel mutations that give rise to antiviral resistance and are uniquely capable of confirming that particular mutations are *necessary* and *sufficient* to confer resistance in multiple strain contexts. Notably, for mutations that confer resistance by altering the function of the NA protein (i.e., versus altering NA expression levels or through epistatic effects), these experiments can be performed using attenuated reassortant strains, though results may not be recapitulated in the context of the wild type strain. *In vitro*, virus-free systems can also be used to discover and validate new mutations that give rise to antiviral resistance by altering the function of the NA protein, but results should be confirmed in the context of the full virus. Given that single mutations are sufficient to confer resistance to NAIs, targeted mutagenesis of antiviral-resistant strains to restore antiviral sensitivity (LoF) is also capable of establishing a causal link between a particular trait and antiviral resistance. However, because this approach relies on the existence of antiviral resistant strains in nature, the ability of this approach to demonstrate that particular markers are conserved across multiple strain contexts is limited relative to GoF approaches. Comparing the sequences of wild type viruses or of patient isolates over the course of antiviral treatment may lead to the identification of mutations that are associated with antiviral resistance, but all hypotheses must be confirmed using targeted mutagenesis (GoF or LoF) to be useful for surveillance. In addition, these approaches are limited to the discovery of antiviral resistance mutations that have already arisen in nature. Computational models can be used to predict mutations that disrupt the interaction between an NAI compound and an antiviral, but predictions must be validated experimentally. Taken together, GoF approaches provide unique benefits for strengthening the predictive value of molecular markers for antiviral resistance, thereby improving their utility for interpreting surveillance data.

Of note, NAI resistance markers that have been shown to be conserved across multiple strain contexts and are currently incorporated into the practice of analyzing surveillance data.[1706] Thus, the benefits of GoF research about molecular markers for antiviral resistance to the practice of surveillance can be realized immediately.

15.7.4.1.3 Utility of Molecular Markers for Antiviral Resistance in Surveillance, Relative to Phenotypic Assays

Resistance to NAIs can be assessed in two ways: through laboratory testing of NAI resistanec using the MUNANA assay or other phenotypic assays and/or by inspecting sequences for the presence of mutations that are known to confer NAI resistance. For characterizing surveillance isolates, both methods have strengths and limitations.

[1706] (2015u) Interviews with influenza researchers and U.S. government representatives involved in influenza surveillance.

The strength of phenotypic assays, relative to predictive approaches, is that phenotypic assays provide a direct readout of antiviral resistance. However, the practice of characterizing the antiviral sensitivity of surveillance isolates through phenotypic assays has several shortcomings. These shortcomings were discussed in detail in Section 15.3.4 and are briefly summarized here. First, the need for viral isolates limits the number of viruses that can be subjected to phenotypic characterization. Second, the composition of viral species present in the original clinical sample changes during isolation, as the most fit viral quasi-species outcompete others. This change is of particular concern for antiviral resistance testing because antiviral-resistant viruses are often less fit than antiviral-sensitive viruses, thus the presence of antiviral resistant strains in mixed infections can be obscured by viral isolation. One government official involved in the pandemic risk assessment process reported that such mixed infections do occur; in one case, the results of antiviral resistance assays were indeterminate, while sequencing of the clinical isolate revealed the presence of both viral genotypes.[1707] Finally, in the event that clinical samples or viral isolates are shipped to WHOCCs for antiviral susceptibility testing, delays stemming from logistical, political, and/or regulatory factors create a lag time between sample collection and phenotypic characterization.

The practice of predicting the antiviral resistance phenotype of surveillance viruses based sequence inspection for molecular markers of antiviral resistance addresses several shortcomings associated with the phenotypic assay approach. In particular, the fact that clinical isolates can be directly sequenced provides several advantages. First, this method provides a direct readout of the viral species present in the sample, avoiding the problem that the composition of viral quasispecies changes during the virus isolation process. Second, following inactivation of virus present in a clinical sample, the sequencing procedure can be carried out under BSL-2 conditions and thus can more feasibly be implemented at NICs and other diagnostic labs in developing countries. Third, whether from clinical samples or virus isolates, sequencing is becoming ever cheaper and easier. As a result, viral genetic sequence data is currently the fastest and most reliable data generated by diagnostic labs in areas where viruses of concern are circulating.[1708] However, most genetic surveillance data is generated by sequencing of viral isolates at WHOCCs, though the number of NICs with sequencing capabilities as well as the number of diagnostic labs (including NICs, WHOCCs, and collaborating labs) that conduct sequencing on clinical samples is increasing. Full realization of the benefits that can be derived from the use of molecular marker data will require an expansion of sequencing capabilities at diagnostic laboratories that comprise the "base" of the influenza surveillance system as well as an increase in the number of clinical samples that are directly sequenced.

The major limitation of using molecular markers is that the predictive value of molecular markers for antiviral resistance is sub-optimal, in part due to a lack of knowledge of the mutational landscape that can give rise to antiviral resistance and to limited knowledge about whether certain markers will convey antiviral resistance in new strain contexts. GoF approaches have potential to address both of those questions, which will improve the utility of molecular marker data to surveillance. Given that several markers have already been shown to be conserved across multiple strain contexts and that only one or two mutations are needed to confer NAI resistance, additional conserved markers for NAI resistance are likely to be identified. Whether similarly conserved antiviral resistance markers can be identified for future antivirals is unknown. Similar to the NAIs, single mutations have been shown to confer resistance to multiple antivirals in development, but the genetic threshold for resistance to some future antivirals may be higher, in which case sequence-based predictions of antiviral resistance become more difficult. Finally, given the inherent uncertainty of sequence-based predictions, researchers and governmental officials involved in the analysis of surveillance data emphasized that predictions should be validated through antiviral resistance assays whenever possible.

[1707] (2015t) Interview with BARDA representative.
[1708] (2015d) Interviews with influenza researchers and government representatives involved in pandemic risk assessments.

Taken together, for characterizing the antiviral sensitivity or surveillance isolates, both phenotypic assays and inspection of sequences for molecular markers of antiviral resistance have strengths and limitations (summarized in Table 15.34). Phenotypic assays provide direct information about the degree of antiviral resistance of a particular strain, but results are delayed relative to sample collection and the properties of viral isolates may not reflect the properties of viral quasispecies present in the original clinical sample. For these reasons, researchers and government officials involved in influenza surveillance value the ability to corroborate phenotypic assay data with sequence-based predictions based on molecular markers of antiviral resistance, particularly when clinical samples can be directly sequenced. As sequencing becomes more common at NICs and other diagnostic laboratories and the number of known, validated markers for NAI resistance rises, the molecular marker approach will take on relatively greater importance. Ultimately, due to the rapidity of sequence-based analysis relative to phenotypic assays, the use of molecular markers may increase capacity to monitor for antiviral resistance.

Table 15.34. Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Surveillance Benefits – Aid Evaluation of the Antiviral Susceptibility of Circulating Seasonal and Animal Influenza Viruses

Approach	Benefits	Limitations

GoF #1: Inspection of sequences for the presence of molecular markers for antiviral resistance	• Improves the accuracy of antiviral susceptibility information
	  o Clinical samples can be directly sequenced
	  o Corroboration of phenotypic assay results increases the robustness of the data
	• Increases the quantity and timeliness of antiviral susceptibility information
	  o As sequencing becomes cheaper and easier, a greater number of viruses may be sequenced than subjected to phenotypic assays
	  o Enables rapid evaluation of antiviral susceptibility, in the event that sequencing data is generated at the site (or in the country) of sample collection
	• Molecular marker data are currently used to interpret surveillance data
	• New data can be incorporated into the process in the immediate term
		• The predictive value of markers for antiviral resistance is currently sub-optimal
		  o Scientific knowledge about the landscape of mutations that can give rise to antiviral resistance is incomplete
		• The use of molecular markers is inherently predictive
		  o Predictions should be validated through phenotypic testing whenever possible
		• Full realization of benefits depends on expanding sequencing capabilities at NICs and increasing the number of clinical samples that are directly sequenced

Alt-GoF #1: Phenotypic characterization of the antiviral susceptibility of surveillance isolates	• Provides a direct readout of antiviral susceptibility	• The need for viral isolates limits the number of viruses that can be characterized • The composition of viruses present in the original clinical samples may change during virus isolation o In mixed infections with antiviral-sensitive and –resistant species, less fit resistant species may be out-competed during the isolation process • Sample shipping delays due to logistical, political, and regulatory factors delay the generation of phenotypic data

15.7.5 Benefits to Decision-Making in Public Health Policy

GoF approaches have potential to benefit surveillance for antiviral resistant strains by improving the practice of using molecular markers for antiviral resistance to infer antiviral resistance from genotype. Surveillance for antiviral resistant strains informs downstream decision-making related to public health practice and policy. Namely, data on the prevalence of antiviral-resistant seasonal strains informs therapeutic recommendations developed by the CDC, and antiviral resistance is one of the risk elements considered in a pandemic risk assessment of an animal influenza virus. This section describes each of these applications, which illustrates the ultimate public health impacts associated with GoF benefits to surveillance. Alternative data sources that inform these public health decisions are also evaluated.

15.7.5.1 Benefits to Decision-Making Related to Seasonal Flu Strains

Two classes of antivirals are FDA-approved for general use against seasonal influenza strains: the adamantanes, which are no longer recommended for therapeutic use due to widespread resistance, and the NAIs, which includes three different small molecule drugs (oseltamivir, zanamivir, and peramivir).[1709,1710] The CDC monitors the prevalence of antiviral resistance among circulating strains to inform their recommendations to clinicians for the use of influenza antivirals. For example, the adamantane class of influenza antivirals (M2 inhibitors) were recommended until 2005, when widespread resistance (>90%) was detected among strains circulating during the 2005 –2006 flu season. This triggered CDC to issue an interim change in their antiviral treatment guidelines, recommending the use of NAIs in lieu of adamantanes.[1711] Although NAI resistance was high during the 2007 – 2008[1712] and 2008 – 2009[1713] seasons (>98% of H1N1 isolates tested), recent outbreak strains have remained susceptible to all three NAIs. However, seasonal strains that are resistant to one and to multiple NAIs have been detected in nature,[1714] sporadic cases of oseltamivir-resistant 2009 H1N1 pandemic virus continue to be detected, and development of resistance to oseltamivir or zanamivir during treatment of seasonal influenza has been documented.[1715,1716,1717] Current antiviral treatment guidelines do not recommend particular NAIs; however, an increase in the prevalence of singly-resistant strains could trigger a recommendation change. As antivirals are most effective when given within 48 hours of symptom onset, the CDC recommends initiating antiviral treatment prior to laboratory confirmation of influenza (i.e., without knowledge of antiviral susceptibility).[1718] Given that, antiviral treatment recommendations based on reliable knowledge about the prevalence of resistance to particular antivirals among circulating strains is essential for the success of therapeutic treatment. Currently, a subset of the influenza viruses that are collected by

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1709 Centers for Disease Control and Prevention. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Accessed: November 4, 2015

1710 (2006) High levels of adamantane resistance among influenza A (H3N2) viruses and interim guidelines for use of antiviral agents--United States, 2005-06 influenza season. *MMWR Morb Mortal Wkly Rep* 55: 44-46

1711 ibid.

1712 Hauge SH *et al* (2009) Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. *Emerg Infect Dis* 15: 155-162

1713 Dharan NJ *et al* (2009) Infections with oseltamivir-resistant influenza A(H1N1) virus in the United States. *JAMA* 301: 1034-1041

1714 L'Huillier AG *et al* (2015) E119D Neuraminidase Mutation Conferring Pan-Resistance to Neuraminidase Inhibitors in an A(H1N1)pdm09 Isolate From a Stem-Cell Transplant Recipient. *J Infect Dis*

1715 Gubareva LV *et al* (2001) Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. Ibid. 183: 523-531

1716 Kiso M *et al* (2004) Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet* 364: 759-765

1717 Hurt AC *et al* (2009) Zanamivir-Resistant Influenza Viruses with a Novel Neuraminidase Mutation. *J Virol* 83: 10366-10373

1718 Centers for Disease Control and Prevention. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Accessed: November 4, 2015

WHOCCs are sent to CDC for antiviral susceptibility testing.[1719] As discussed above, phenotypic assay results are often corroborated by sequence inspection for the presence of molecular markers associated with antiviral resistance. As sequencing becomes more common at NICs and other diagnostic laboratories and the number of known, validated markers for NAI resistance rises, the rapidity of the molecular marker approach may expand the number of surveillance viruses that can be phenotypically characterized. This expansion will provide a stronger foundation for antiviral treatment recommendations and may enhance detection of rare antiviral-resistant variants to increase awareness.

15.7.5.2 Benefits to Decision-Making Related to Pandemic Influenza

Antiviral resistance is one of the risk elements considered in pandemic risk assessments of animal influenza viruses, which inform downstream decision-making about investments in pre-pandemic preparedness initiatives (discussed in detail in Section 16.3.5.2). This section analyzes the value of antiviral resistance information relative to other types of information considered in risk assessments, as well as particular contributions of antiviral resistance information to downstream decision-making.

For pandemic risk assessments, the antiviral resistance risk element does not contribute to the likelihood that an animal virus will emerge to efficiently infect and transmit in humans and moderately contributes to the assessment of the expected consequences of an emergence event. For example, in a recent risk assessment of avian H7N9, avian H1N1, and swine H3N2v viruses, the antiviral resistance element was worth less than the disease severity, population immunity, and extent of human infections risk elements (approximately one-third as much as the most highly weighted disease severity element).[1720] Stakeholders involved in the pandemic risk assessment process emphasized that antiviral resistance does not increase risk a priori but rather is important when coupled to other factors indicative of increased pandemic potential, such as a high number of human infections or enhanced transmissibility or virulence in ferrets. In this case, the observation of antiviral resistance may trigger USG representatives to initiate the process of applying for Emergency Use Authorization (EUA) from the FDA for antivirals in development, to ensure that effective antivirals will be available if the strain under evaluation spreads to cause a pandemic. Importantly, when evaluating antiviral resistance, stakeholders consider both phenotypic and genetic data, given the caveats associated with both types of data (discussed above). Additionally, the ability to conduct a rapid risk assessment based on sequence data, by inspection of sequences for molecular markers of antiviral resistance, is valuable when strains first emerge and sequences are published prior to the receipt of viral isolates. For example, in 2013, the observation that the sequences of early clinical isolates of avian influenza H7N9 in China contained molecular markers previously shown to confer to oseltamivir and zanamivir informed BARDA's decision to move forward with the decision to initiate the EUA process for antivirals in development.[1721] Given the two-week lag time between publication of the H7N9 sequence and additional time needed to conduct antiviral sensitivity testing, the ability to use molecular markers to infer antiviral resistance phenotypic from genotype provided a several week head start on the EUA process. There is no set time frame for approval of an EUA, but approval can be granted within days if the FDA has already reviewed the relevant data on the MCM (submitted in advance as a "pre-EUA" package).[1722] For example, the FDA issued an EUA for peramivir in October 2009 in response to the H1N1 influenza pandemic three days after the request and recently issued an EUA for the DoD EZ1 rRT-PCR Ebola diagnostic in August 2014 one day following the request. In both cases, the FDA had worked with their government partners on pre-EUA packages in advance of the requests. Thus, a several

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[1719] Centers for Disease Control and Prevention. Antiviral Drug Resistance among Influenza Viruses. http://www.cdc.gov/flu/professionals/antivirals/antiviral-drug-resistance.htm. Last Accessed: November 4, 2015

[1720] Cox NJ *et al* (2014) Pandemic preparedness and the Influenza Risk Assessment Tool (IRAT). *Current topics in microbiology and immunology* 385: 119-136

[1721] (2015t) Interview with BARDA representative.

[1722] (2015m) Personal communication from FDA representative

week head start on the process could significantly impact the timing of availability of antivirals in the event of a pandemic.[1723]

Taken together, this suggests that the ability of GoF benefits to surveillance for antiviral-resistant viruses to contribute to the overall risk assessment score is moderate and that the ability the infer antiviral resistance phenotype based on genotype may provide a valuable head start on the EUA process for antivirals in development when novel strains that are resistant to licensed antivirals emerge.

15.7.6 GoF Benefits to the Development of vaccines

15.7.6.1 Vaccine Development Benefit: Targeted Mutagenesis to Remove Antiviral Resistance Markers from Vaccine Viruses

Vaccine viruses comprise the HA and NA genes from the wild type strain of interest and the remaining six genes from a vaccine backbone virus such as PR8. Mutations that confer resistance to NAIs, the one approved class of influenza antivirals that are recommended for use in the US, arise in the NA gene.[1724,1725,1726,1727] If the wild type NA gene contains conserved markers for NAI resistance, these markers can be removed through targeted deletion or mutagenesis to increase the safety of the vaccine production process. (Of note, most influenza vaccines produced in the US are inactivated, thus whether a vaccine strain is sensitive or resistant to antivirals has no impact on the safety of the vaccine itself.) For example, this strategy was used for production of a pre-pandemic H7N9 vaccine in 2013.[1728] By sequence inspection, clinical isolates from the first few cases of H7N9 were found to contain the R292K mutation, which had previously been shown to reduce resistance to multiple NAIs.[1729] As a result, this mutation was eliminated from the NA gene of the vaccine virus used for production of clinical lot material.[1730] Of note, candidate vaccine viruses (CVVs) are not typically tested for antiviral sensitivity as part of the routine set of characterization assays performed prior to release of CVVs to manufacturers.[1731,1732,1733]

[1723] Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

[1724] Baz M *et al* (2010) Effect of the neuraminidase mutation H274Y conferring resistance to oseltamivir on the replicative capacity and virulence of old and recent human influenza A(H1N1) viruses. *J Infect Dis* 201: 740-745

[1725] Kaminski MM *et al* (2013) Pandemic 2009 H1N1 influenza A virus carrying a Q136K mutation in the neuraminidase gene is resistant to zanamivir but exhibits reduced fitness in the guinea pig transmission model. *Journal of virology* 87: 1912-1915

[1726] Baz M *et al* (2006) Characterization of Multidrug-Resistant Influenza A/H3N2 Viruses Shed during 1 Year by an Immunocompromised Child. *Clin Infect Dis* 43: 1555-1561

[1727] Hai R *et al* (2013) Influenza A(H7N9) virus gains neuraminidase inhibitor resistance without loss of in vivo virulence or transmissibility. *Nat Commun* 4

[1728] (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

[1729] Hai R *et al* (2013) Influenza A(H7N9) virus gains neuraminidase inhibitor resistance without loss of in vivo virulence or transmissibility. *Nat Commun* 4

[1730] (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

[1731] Vaccine response to the avian influenza A(H7N9) outbreak
- step 1: development and distribution of candidate vaccine viruses.
http://www.who.int/influenza/vaccines/virus/CandidateVaccineVirusesH7N9_02May13.pdf. Last Update Accessed September 14, 2015.

[1732] Update of WHO biosafety risk assessment and guidelines for the production and quality control of human influenza vaccines against avian influenza A(H7N9) virus.
http://www.who.int/biologicals/areas/vaccines/influenza/biosafety_risk_assessment_10may2013.pdf. Last Update Accessed September 14, 2015.

[1733] (2015q) Current practices in influenza vaccine production. Interview with Industry or Federal Government Representative with Expertise in Influenza Vaccine Production.

The strengths and limitations of GoF and alt-GoF approaches for identifying antiviral resistance markers that can be removed from vaccine viruses are summarized in Table 15.35. Both GoF and alt-GoF approaches can be used to identify mutations that confer antiviral resistance to currently circulating influenza strains. GoF approaches are relatively more efficient and effective for the discovery of new antiviral resistance markers but may uncover mutations that do not yet exist in nature, which is not relevant for this application because vaccine viruses are based on wild type viruses. The FDA, which must approve of all vaccine strains that are used for large-scale production, prefers that the HA and NA genes of a vaccine strain are as close to the wild type strain as possible.[1734] As a result, markers without causal links to antiviral resistance across multiple strain contexts may not be approved for this application (though approval would depend on the level of manipulation and would be considered on a case-by-case basis). Both GoF and alt-GoF approaches can provide this information. GoF approaches, namely targeted mutagenesis of antiviral-sensitive strains to introduce mutations expected to confer antiviral resistance, can be used to demonstrate that a particular mutation (or set of mutations) is *necessary* and *sufficient* to confer resistance. Alternative approaches, namely targeted mutagenesis of antiviral-resistant strains to introduce mutations expected to restore antiviral sensitivity, can be used to demonstrate that a particular amino acid or set of amino acids are *necessary* for antiviral resistance. As the ultimate goal is to restore antiviral sensitivity to vaccine strains harboring NAI-resistant NA genes, either approach is equally suitable for identifying molecular markers linked to antiviral resistance for this purpose.

Table 15.35. Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Benefits to Vaccine Development: Targeted Mutagenesis to Remove Antiviral Resistance Markers from Vaccine Viruses		
Approach	**Benefits**	**Limitations**
GoF Experimental Approaches: • Serial passaging of viruses in the presence of therapeutic • Genetic modification of antiviral-sensitive strains to introduce genetic traits expected to confer antiviral resistance	• Efficient and effective approaches for discovering new mutations that confer antiviral resistance to currently circulating strains • Targeted GoF can be used to demonstrate that particular mutations are necessary and sufficient to confer antiviral resistance across multiple strain contexts	• May uncover antiviral resistance mutations that do not yet exist in nature, which are not relevant for this application
Alt-GoF Experimental Approaches: • Genetic modification of antiviral-resistant strains to introduce traits expected to restore antiviral sensitivity • Other approaches (see table 15.32)	• Discover new mutations that confer antiviral resistance to currently circulating strains • Targeted LoF can be used to demonstrate that particular mutations are necessary for antiviral resistance across multiple strain contexts	• Approaches are less efficient and effective for the discovery of novel antiviral resistance markers than GoF approaches

15.7.7 GoF Benefits to the Development of Therapeutics

Only two classes of FDA-approved antivirals are approved for use in the US: the adamantanes, which inhibit the viral M2 protein, and the neuraminidase inhibitors (NAIs), which include zanamivir (Relenza), oseltamivir (Tamiflu), and peramivir (Rapivab).[1735] The adamantanes are no longer recommended for use

[1734] Ibid.

[1735] CDC. Influenza Antiviral Medications: Summary for Clinicians. http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm. Last Update November 3, 2015. Accessed November 28, 2015.

due to widespread resistance. Single mutations are sufficient to confer resistance to one or multiple NAIs and have been observed in nature, though NAI-resistance mutations are not yet widespread. Moreover, the NAIs exhibit limited efficacy, especially if administered more than 48 hours after symptom onset.[1736] Thus, there is an urgent need for the development of new therapeutics against influenza viruses.[1737]

15.7.7.1 Therapeutic Development Benefit 1: Inform Development of Therapeutic Candidates

Given the high mutation rate of influenza viruses, viruses can readily acquire mutations to many therapeutics. Screening potential therapeutics based on how readily antiviral resistance emerges provides one mechanism for differentiating between therapeutic candidates based on their likely field efficacy. Prior to field deployment of a therapeutic, serially passaging viruses in the presence of therapeutic, a GoF approach, is uniquely capable of determining whether and how readily resistance arises. Furthermore, as resistance is expected to arise in human populations following deployment of the therapeutic, determining whether resistance enhances the infectivity, transmissibility, or virulence of a virus is an important aspect of safety testing of the therapeutic candidate. Taken together, GoF approaches are critical for testing the potential efficacy and safety of new therapeutic candidates.

15.7.7.2 Therapeutic Development Benefit 2: Facilitate Regulatory Approval of Therapeutic Candidates

The first step in the licensure process for new drugs involves submission of an Investigational New Drug (IND) application to the FDA's Center for Drug Evaluation and Research (CDER). CDER recommends that several types of nonclinical studies are conducted before starting Phase I clinical studies, including determination of the drug's mechanism of action, *in vitro* selection of resistant viruses to the investigational product, and the genotypic and phenotypic characterization of resistant viruses.[1738] Mechanism of action studies should demonstrate the investigational product's ability to specifically inhibit viral replication or virus-specific function and should establish the site of the product's action.

GoF approaches have the potential to support two aspects of an IND application for therapeutics in development: (1) determination of the mechanism of action of a therapeutic and (2) the *in vitro* selection of resistant viruses.

15.7.7.2.1 Determining the Mechanism of Action of a Therapeutic

The FDA recommends that a drug's mechanism of action be "well-characterized" prior to the start of Phase I clinical trials and requests this information as a component of an IND application, the first step of the licensing process.[1738] The influenza field is pursuing multiple strategies for developing new therapeutic candidates, including the deliberate design or selection of therapeutics targeting specific viral or host proteins and high-throughput screening of libraries of small molecules to identify compounds that reduce viral replication *in vitro*. In the former case, the drug target of the therapeutic candidate is known, while in the latter case, the therapeutic target is unknown. GoF approaches can be used to gain insight into the mechanism of activity of therapeutics that directly target virus proteins, thus benefitting the

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[1736] CDC. Use of Antivirals. Background and Guidance on the Use of Influenza Antiviral Agents http://www.cdc.gov/flu/professionals/antivirals/antiviral-use-influenza.htm. Last Update February 25, 2015. Accessed November 28, 2015.

[1737] (2015h) Interviews with influenza researchers.

[1738] Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

[1739] Ibid.

development of new drugs. Below, the benefits of GoF approaches, relative to alternative experimental approaches, for the determination of antiviral mechanisms in both of these scenarios is evaluated.

Benefits and Limitations of GoF Approaches

Passaging viruses in cells in the presence of a therapeutic is a classic method for generating viruses that can **evade the inhibitory action of the therapeutic**, thus constituting a GoF approach. Viruses are then sequenced to identify mutations that arose, and if multiple mutations are present, mutations are re-introduced into the parental strain individually and in combination to identify the minimal set of mutation(s) that are necessary and sufficient to confer antiviral resistance. Understanding which viral protein or proteins mutate in order for the virus to escape inhibition suggests those proteins are targeted by the therapeutic, and the site and phenotypic consequences of the mutations may provide insight into the mechanism of antiviral activity. Together, this information provides a foundation for follow-up structural, biochemical, and cell biological assays investigating the mechanism of antiviral activity. A major strength of this approach is that it can be applied to any type of therapeutic, including therapeutics with known targets (but unknown mechanisms of action) and therapeutics with unknown targets. However, elucidating the mechanisms of antiviral activity based on indirect observations about antiviral resistance can be challenging. For example, mutations may arise in proteins that are not directly targeted by the therapeutic, or the phenotypic consequences of mutations may be unclear.[1740,1741,1742] Additionally, if the drug targets a host protein, this approach provides indirect information about its mechanism of activity, which must be inferred based on prior knowledge of virus-host interactions.

Benefits and Limitations of Alt-GoF Approaches

Therapeutic candidates that are identified through high-throughput screens may attenuate viral replication by directly targeting viral proteins or by indirectly targeting host proteins. For that reason, emergence of resistance studies, which investigate potential viral targets, are usually complemented by high-throughput RNAi screens targeting host proteins, to investigate potential host targets. Specifically, the fact that knockdown of a particular host protein impedes the drug's ability to inhibit viral replication suggests that that protein or that signaling pathway may be targeted by the therapeutic. Though an informative strategy for the study of therapeutics targeting host proteins, high-throughput RNAi screens provide minimal information about potential viral targets of therapeutics. Viral targets must be inferred based on prior knowledge of virus-host interactions, which is likely to be challenging. Furthermore, because this kind of indirect information does not provide insight into antiviral mechanisms, this host-focused approach is of limited value for the study of therapeutics with known viral targets.

If the therapeutic target of a drug is known, analyzing the crystal structure of the viral target in complex with the antiviral compound (or mAb) can provide insight into the compound's mechanism of activity.[1743,1744] This approach is particularly useful for therapeutics that directly bind to and inhibit the activity of a viral protein. Though X-ray crystallography is appealing for its potential to provide direct information about the interaction between an antiviral and its target, inferring how that interaction affects a process in the viral life cycle may be difficult from such a static snapshot. In addition, this approach is less suitable for investigating therapeutics that target a protein-protein or protein-nucleic acid complex

1740 Wensing AM *et al* (2014) 2014 Update of the drug resistance mutations in HIV-1. *Topics in antiviral medicine* 22: 642-650

1741 Staschke KA *et al* (1995) Molecular basis for the resistance of influenza viruses to 4-guanidino-Neu5Ac2en. *Virology* 214: 642-646

1742 Blick TJ *et al* (1998) The interaction of neuraminidase and hemagglutinin mutations in influenza virus in resistance to 4-guanidino-Neu5Ac2en. Ibid. 246: 95-103

1743 Prabakaran P *et al* (2006) Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. *The Journal of biological chemistry* 281: 15829-15836

1744 Ratia K *et al* (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences of the United States of America* 105: 16119-16124

(either a virus-host complex or a virus-virus complex), either to inhibit the function or block the formation of the complex. The relevant interaction partner may be unknown, or recombinantly producing and crystallizing the protein complex may be difficult. Critically, because of the high level of effort required for X-ray crystallography, it is not a feasible approach for simply screening the potential viral targets of an unknown antiviral.

Photoaffinity cross-linking represents an alternative approach for identifying the binding site of a drug with a known target. In brief, this approach relies on the use of a "photoaffinity analogue" of the candidate therapeutic, which is synthesized to contain a photosensitive group (e.g., an azide) and a radioactive isotope (e.g., tritium, ^{3}H).[1745] After treating the viral protein with the photoaffinity analog, the sample is irradiated with UV light, triggering the photosensitive group to form a covalent bond with the viral enzyme. Analytical techniques such as mass spectrometry can then be used to identify the labeled amino acid residues in order to determine the drug's binding site. This technique shares strengths and weaknesses with X-ray crystallography. Namely, photoaffinity cross-linking is useful for small molecule drugs that directly bind to and inhibit the activity of a viral protein and does not require prior knowledge of the location of the drug binding site.[1746] However, inferring the mechanism of antiviral activity based on knowledge about the drug-virus protein interaction may be difficult, and the approach is less suitable for studying therapeutics that target a protein-protein or protein-nucleic acid complex (either a virus-host complex or a virus-virus complex).

Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

The strengths and limitations of GoF and alt-GoF approaches that can provide insight into the mechanism of action of a candidate therapeutic are summarized in Table 15.36. Taken together, serial passaging of a virus in the presence of therapeutic to discover mutations that confer resistance, a GoF approach, is uniquely capable of identifying the viral target of a novel therapeutic with an unknown mechanism of action. Given that researchers are undertaking unbiased screens to identify candidate therapeutics that inhibit viral replication, this represents a valuable benefit for the development of new influenza therapeutics. For therapeutics with known viral targets, this information about resistance mutations can provide foundational information to guide follow-up structural, cell biological, and biochemical studies investigating the mechanism of action of the therapeutic. Although crystallography and photoaffinity cross-linking can also provide insight into the antiviral mechanisms of therapeutics that directly bind to and inhibit virus proteins, inferring mechanistic information based on static information about the virus-antiviral complex may be difficult. In these cases, knowledge about mutations that confer resistance, generated through GoF approaches, provides an additional source of information that can be used to generate testable hypotheses about mechanism of antiviral activity. Finally, the identification of host factors that are required for antiviral activity is a critical aspect of examining therapeutics with unknown targets. Though solely using host-focused approaches to elucidate the antiviral mechanism of a therapeutic that targets the virus would be difficult, this information complements GoF approaches to strengthen the evidence base for the drug's mechanism of action.

[1745] Cohen KA *et al* (1991) Characterization of the binding site for nevirapine (BI-RG-587), a nonnucleoside inhibitor of human immunodeficiency virus type-1 reverse transcriptase. *The Journal of biological chemistry* 266: 14670-14674

[1746] Hamouda AK *et al* (2014) Photoaffinity labeling of nicotinic receptors: diversity of drug binding sites! *Journal of molecular neuroscience : MN* 53: 480-486

Table 15.36. Summary of the Benefits of GoF Approaches That Lead to Evasion of Therapeutics

Benefits to Therapeutic Development: Identify the Mechanism of Action of a Candidate Therapeutic		
Approach	**Benefits**	**Limitations**
GoF #1: Serial passaging of viruses in the presence of therapeutic [1]*	• Identify the *viral* protein target of a candidate therapeutic with an unknown target • Provide insight into the mechanism of action of the therapeutic through the identification of mutations that confer resistance	• Elucidating the mechanism of action of a therapeutic based on indirect information about resistance mutations may be difficult o Resistance mutations may arise in non-target proteins, confounding interpretation of results • Not suitable for identifying the targets of therapeutics that target host proteins
Alt-GoF #1: RNAi screen targeting host proteins to identify host proteins that are critical for the antiviral activity of a therapeutic	• Identify the *host* protein target of a candidate therapeutic with an unknown target	• Provides indirect information about the viral protein targets of a therapeutic
Alt-GoF #2: Analyze the crystal structure of a therapeutic in complex with its viral protein target	• Provides direct information about the interaction between a therapeutic and its viral protein target o May provide insight into the mechanism of antiviral activity	• Limited to the study of therapeutics with known targets • Inferring mechanism of activity based on static information about the therapeutic-viral protein interaction may be difficult • Approach may not be suitable for the study of therapeutics that target protein-protein protein-nucleic acid complexes
Alt-GoF #3: Photo-affinity crosslinking	• Provides direct information about the binding site of a therapeutic on its viral protein target • May provide insight into the mechanism of antiviral activity	• Limited to the study of therapeutics with known targets • Inferring mechanism of activity based on static information about the therapeutic binding site may be difficult • Approach may not be suitable for the study of therapeutics that target protein-protein protein-nucleic acid complexes

**Numbers in brackets reference specific experimental approaches in the landscape tables (Supplementary Information).*

15.7.7.2.2 Determining the Genetic Threshold for Resistance Development

Prior to the conduct of clinical trials and to support an IND application, the FDA recommends conducting *in vitro* studies for **selection of resistance to a therapeutic** in order to determine the genetic threshold for resistance development (i.e., how many mutations are needed to acquire resistance). Specifically, the FDA recommends passaging the virus in the presence of therapeutic, followed by sequencing of emergent resistant viruses and phenotypic characterization of resistant viruses.[1747] Selection for resistance studies should be repeated multiple times to determine if the same or different patterns of resistance mutations develop, as well as to determine how the concentration of the therapeutic impacts how readily resistance develops. These studies constitute GoF approaches. The FDA guidance does not suggest any alternative approaches that could provide similar information. In fact, prior to deployment of the therapeutic and the emergence of resistant viruses in nature, no alternative approaches can provide this information. Thus, GoF approaches that lead to the generation of viruses that are resistant to therapeutics in development are **essential** for the licensing of new therapeutics.

15.7.7.3 Therapeutic Development Benefit 3: Inform Guidelines for Use of Therapeutics

The therapeutic regimen, including therapeutic dose and the use of combination therapies, may influence whether and how readily antiviral resistance arises. Given that influenza viruses readily acquire resistance to NAIs (i.e., upon acquisition of one or two mutations), influenza researchers cited a lack of knowledge about the potential utility of combination therapies as a critical gap in public health preparedness for influenza epidemics and pandemics.[1748] In addition, understanding whether antiviral resistance arises more readily or differently in at-risk populations, such as obese or immunocompromised people, in either scenario can provide information that further refines therapeutic guidelines. GoF approaches can address each of these questions.

GoF approaches that lead to the development of viruses with **resistance to therapeutics in development** can be used to evaluate the relationship between emergence of resistance and therapeutic dosage or the administration of multiple therapeutics in combination. First, serial passaging of virus in animals dosed with varying amounts of the therapeutic provides insight into the dose-dependence of the emergence of resistant viruses. Because host-dependent factors, such as the rate of metabolism or clearance of the therapeutic, influence the concentration of therapeutic the virus experiences, conducting passaging studies in animals provides more relevant information than *in vitro* passaging studies. Second, serial passaging of virus in cells or in animals in the presence of multiple mAbs (or other types of therapeutics) can be used to determine how readily resistance arises in response to combination versus single therapies. Although *in vitro* selection studies are useful for screening different combinations of therapeutics, because of the role of bioavailability and other host-dependent factors on antiviral efficacy, all promising combination therapies should be validated through *in vivo* passaging experiments. In both cases, serial passaging of virus in mouse models for at-risk populations (e.g., immunocompromised mice or obese mice) provides additional information about the extent to which the likelihood of resistance or patterns of resistance mutations vary depending on host factors, which may inform therapeutic guidelines for specific at-risk populations. No alternative approaches are capable of providing similar information about the dose-dependence of resistance or whether combination therapies lead to resistance less readily than individual therapies.

[1747] Food and Drug Administration. Guidance for Industry: Antiviral Product Development - Conducting and Submitting Virology Studies to the Agency. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf. Last Update June 2006. Accessed 14 October 2015.

[1748] (2015h) Interviews with influenza researchers.

Taken together, GoF approaches that lead to the generation of viruses that are resistant to therapeutics in development are uniquely capable of determining the therapeutic dose that is least likely to lead to the acquisition of antiviral resistance as well as determining whether combination therapies better prevent the emergence of resistant viruses than individual therapies. Both types of information benefit the development of therapeutic strategies that will be effective for a longer period of time in the field.

15.8 Influenza Viruses: Detailed Analysis of the Benefits of GoF Research Involving Reassortment

15.8.1 Overview of Influenza GoF Landscape

This assessment describes the benefits of GoF experimental approaches that aim to assess the genetic compatibility and fitness of viruses following reassortment. While the phenotypic consequences of reassortment events between two viruses cannot be predicted with certainty, reassortant strains may exhibit enhanced fitness, pathogenicity, and/or transmissibility relative to one or both parental strains. (Notably, reassortant strains may also display *reduced* fitness, pathogenicity, and/or transmissibility relative to parental viruses.) In this section, we provide an overview of GoF approaches that can be used to assess the reassortment potential between two viruses and describe the scientific outcomes of each approach. Each approach will be discussed in more detail in the context of our detailed analysis of the benefits of GoF and alt-GoF research that can provide insight into the genetic compatibility and reassortment potential of multiple viruses, below.

15.8.1.1 Targeted Reassortment by Combining Viral Gene Segments from Two or More Viruses to Generate Viable Reassortant Viruses

Targeted reassortment of virus gene segments from two or more wild type virus isolates followed by characterization of fitness in cell culture or in representative animal models is used to assess genetic compatibility. This approach is in part performed to evaluate the genetic compatibility and viability of a *single* reassortant virus, which can inform the understanding of the mechanisms underlying genetic compatibility between virus gene segments across virus strains and subtypes. For example, a reassortant virus compromised of virus gene segments sharing homology to the 1918 H1N1 pandemic virus from eight different wild type avian isolates was generated to demonstrate that some 1918-like avian viruses circulating in nature (which reassort frequently) are genetically compatible.[1749]

15.8.1.2 Forward Genetic Screen to Identify Viable Reassortant Viruses

Forward genetic screens involve the generation of a panel of clonal recombinant viruses by comprehensive reassortment of parental gene segments from two viruses (i.e., all or many possible gene combinations), followed by characterization of the fitness of reassortants in appropriate mammalian model systems. Follow-up studies may be performed to evaluate pathogenicity, infectivity, and/or transmissibility of viable reassortants. This approach is performed to evaluate viability and genetic compatibility of reassortant viruses, which provides a foundation for studies investigating mechanisms governing reassortment and informs the potential for reassortant viruses to emerge in nature and the potential public health consequences of such an emergence event.

[1749] Watanabe T *et al* (2014) Circulating avian influenza viruses closely related to the 1918 virus have pandemic potential. *Cell Host Microbe* 15: 692-705

15.8.1.3 Non- Targeted Reassortment Using Reverse Genetics to Select for Viable Reassortant Viruses

In this approach, reassortants are generated using reverse genetics to mix viral gene segments of two wild type viruses (i.e., mix up to 16 gene segments in total) in the context of cell culture or animal models. Use of cell culture model systems involves the transient transfection of viral gene segments, while the *in vivo* method involves the inoculation of ferrets with transiently transfected cells, followed by viral reassortment *in vivo*. Both approaches are followed by limited passaging to select for viable reassortants. Clonal isolates that emerge are then genotyped to identify their gene composition. This approach provides insight into viable gene reassortment combinations as well as the relative fitness of reassortants under selection pressures, which informs the potential and likelihood of reassortment emergence in nature.

15.8.1.4 Co- Infection to Select for Viable Reassortant Viruses

In this approach, cultured cells or representative animal models are co-infected with two different wild type viruses, followed by genotyping of clonal isolates that emerge during co-infection. This approach determines the viability of various gene reassortment combinations *and* the relative fitness of reassortants under selection pressures, which can inform the potential and likelihood of emergence in nature.

15.8.2 Overview of the Potential Benefits of GoF Experiments Involving Reassortment

Here we evaluate whether any of the GoF Influenza approaches have the potential to benefit each of the general benefit areas described in the NSABB's "Framework for Conducting Risk and Benefit Assessments of Gain of Function Research." We also describe additional benefit areas we identified during our research. Each potential benefit will be analyzed in detail below.

15.8.2.1 Scientific Knowledge

GoF approaches benefit scientific knowledge by providing insight into the reassortment potential between different virus strains, including human seasonal and animal strains, two different human seasonal strains, two different animal strain sub-types, and two different animal strains within the same sub-type. Specifically, GoF approaches can determine the genetic compatibility between virus strains and the phenotypic properties of reassortant viruses, including fitness, transmissibility, and virulence.

15.8.2.2 Surveillance

GoF approaches may benefit surveillance for reassortant viruses. Specifically, information about the phenotypic properties of reassortant viruses may inform assessment of the risks posed by reassortant viruses detected in nature.

15.8.2.3 Vaccines, Therapeutics, and Diagnostics

GoF-derived information about the reassortment potential of two different viruses is not relevant for the development of vaccines or therapeutics.

As existing influenza diagnostics are not equipped to rapidly screen and detect reassortants, information about reassortants with phenotypic properties of concern could, in principle, guide development of diagnostics to detect those reassortants. However, GoF approaches do not provide insight into the likelihood that reassortment will occur in nature, which is a function of complex ecological factors that govern the likelihood of co-infections. The likelihood of reassortment is also a critical factor for the design of targeted diagnostics for reassortant viruses (i.e., there is no need to design diagnostics for rare

reassortant events). For this reason, GoF approaches are unlikely to trigger the development of new diagnostics independently of the observation of co-infection or reassortment events occurring in nature.

15.8.2.4 Informing Policy Decisions

GoF reassortment studies have potential to benefit two aspects of public health practice and policy. First, the results of reassortment studies may stimulate risk mitigation activities to limit the potential for "risky" co-infections to occur in nature in human and/or animal hosts (i.e., those co-infections that could give rise to reassortant viruses with risky properties). Second, reassortment studies may inform pandemic risk assessments of circulating animal influenza viruses, which guide downstream decision-making about pre-pandemic vaccine development and other pandemic preparedness initiatives.

15.8.2.5 Economic Benefits

No economic benefits of GoF reassortment studies were identified.

15.8.3 Benefits of GoF to Scientific Knowledge

Here, the ability of GoF approaches to address a key outstanding question related to the reassortment of influenza viruses in humans and other host species is evaluated:

- What is the potential for reassortment between two influenza virus strains?
  - Are two influenza viruses genetically compatible?
  - What is the relative fitness of reassortants that may affect the likelihood of their emergence under selection in a host?
  - How do selection pressures influence reassortment?

Reassortment involves the exchange of one or more complete virus gene segments between two different viruses during the co-infection of a single cell. The process of reassortment contributes to influenza virus evolution and viral diversity by allowing the rapid exchange of genetic and phenotypic properties under selection pressure and can result in viruses that display enhanced fitness, immune evasion and antigen escape, and resistance to antivirals.[1750] Notable examples of the role of reassortment in influenza virus biology include the reassortment of seasonal and animal influenza viruses leading to the emergence of human pandemic viruses with minimal population immunity.[1751] Considerable gaps in knowledge remain about the biology and prevalence of reassortment in nature within and across host populations. Accordingly, whether such events will occur and will lead to the generation of viruses with enhanced fitness, pathogenicity, and/or transmissibility is not understood. Although many of the unknowns regarding reassortment fall outside the scope of GoF research, GoF approaches can be used to understand whether two viruses are genetically compatible, which provides a foundation for follow-up studies investigating the mechanistic basis of genetic compatibility. These studies include efforts to identify how multiple viral gene segments cooperate to shape other viral phenotypes such as replicative fitness and efficient cell entry and exit. (We note that follow-up studies that are focused on characterizing the pathogenicity or transmissibility of reassortant viruses are covered separately, in the relevant GoF phenotype Sections, 15.3 and 15.4.)

[1750] Steel J, Lowen AC (2014) Influenza A virus reassortment. *Current topics in microbiology and immunology* 385: 377-401
[1751] Scholtissek C (1994) Source for influenza pandemics. *European journal of epidemiology* 10: 455-458

15.8.3.1 Scientific Knowledge Gap: What Is the Potential/Capability for Reassortment Between Two Influenza Virus Strains?

15.8.3.1.1 Benefits and Limitations of GoF Approaches

Several GoF approaches can lead to the generation of reassortant viruses:

- Targeted reassortment to generate a virus comprised of gene segments from two or more wild type isolates,
- Forward genetic screens involving comprehensive reassortment to generate a panel of clonal viral isolates followed by assessment of fitness in cell culture or representative animal models,
- Non-targeted reassortment involving gene segments from two different viruses to generate a mixed population of reassortant viruses, followed by selection for compatible virus genotypes in cell culture or representative animal models, and
- Co-infection of cell culture or representative animal models with two different viruses to select for compatible virus genotypes.

Collectively, these approaches definitively demonstrate whether reassortment can occur between wild type viruses and enable the identification of reassortment gene combinations that permit replication in *in vitro* or *in vivo* model systems. This provides insight into the genetic compatibility of virus gene segments. For the targeted reassortment approach, viral gene segments are selected based on a property of interest (e.g., homology to a human pandemic virus) to answer a specific question about the genetic compatibility between two or more viruses, which differs from the other GoF approaches that more broadly query the range of reassortment combinations that are possible between two viruses. Because forward genetic screens individually test every possible gene combination between two viruses, this GoF approach can assess the viability and fitness of *each* viral clone that may otherwise be missed with selection based approaches (below) in which more fit clones outcompete. However, the outcomes associated with forward genetic screens are independent of the selection pressures that shape reassortment potential and viral population diversity and therefore may not fully represent the likelihood of reassortants emerging.

The use of non-targeted reassortment by transfection of cell culture model systems with gene segments from two separate viruses to select and identify emergent reassortants presents several different advantages. First, this approach provides insight into how host pressures and competition among reassortants shapes outcomes. Importantly, this approach can evaluate selection pressures independent from the requirement of co-infection of the same host cell and is therefore not impacted by differences in the receptor specificity and efficiency of cell entry of parental viruses. Second, the ability to selectively remove a single gene segment that may otherwise outcompete or skew virus populations enables assessment of the compatibility of many gene segment combinations, relative to the co-infection approach. Similar to the non-targeted reassortment approach, the co-infection approach provides insight into how the host pressure and competition impact selection. A major benefit of this approach is that it mimics the natural scenario under which reassortment can occur. However, in the event that two viruses of interest display different tissue and cell tropism or significant disparity in fitness or infectivity, this approach permits study of a limited number of reassortment combinations. For all three approaches, the use of *in vivo* animal models for reassortment studies provides more relevant information due to the complexity of host selection pressures relative to cell culture models. All GoF approaches described here depend on whether the mechanisms and selection pressures underlying fitness and reassortment in cell

culture or animal models are representative of those in humans and whether the genetic compatibility observed for the select number of strains analyzed is generalizable in other virus contexts. Moreover, the use of the methods above may not capture the dynamics of co-infection and reassortment in nature, which are likely dependent on the time and exposure to influenza viruses in addition to the factors discussed above like disparities in fitness among viral isolates in humans.

15.8.3.1.2 Benefits and Limitations of Alt-GoF Approaches

A select number of alt-GoF approaches can be used to analyze the reassortment potential of two different viruses. Analyzing the sequences of human and animal surveillance isolates to detect reassortment events can provide insight into the occurrence and prevalence of reassortment in nature. This approach includes sequence inspection for several different types of reassortment events, involving:

- Two different human seasonal virus sub-types (e.g., H1N1 and H3N2),
- Human or animal virus strains within the same sub-type (e.g., different clades of H3N2),
- Human and animal viruses (e.g., human seasonal H3N2 and swine-origin H1N1), and
- Two different animal virus sub-types (e.g., H9N2 and H7Nx).

Analysis of both animal and human isolates provides information that is applicable to a broad number of strains, and the analysis of human isolates provides information about reassortment potential that is directly relevant to human populations. However, this approach is significantly limited by the quality and availability of existing genetic surveillance data. Reassortment events are most commonly identified through individual phylogenetic analysis of each viral gene segment to identify origin and ancestry.[1752] This requires full genome sequences and large sequence databases for effective determination of phylogenetic ancestry, which are not always available. Furthermore, in cases of low genetic diversity between parental strains, distinguishing between mutations and reassortment events may be difficult, while in cases of high viral diversity between parental strains, distinguishing between reassortant and wild type gene segments may be difficult. In addition, this analysis is limited to the study of reassortant viruses that have evolved (and have been subsequently detected) in nature.

A second type of alt-GoF approach involves the analysis of viral isolates from humans or animals that have been co-infected with two influenza viruses. This approach can determine whether reassortment has occurred and also may provide insight into the genetic compatibility of various gene combinations, as well as host selection pressures that shape the outcome of reassortment events. That analysis of human and animal isolates provides information that is directly relevant to reassortment potential in nature is a strength of this method. However, this approach is also subject to significant limitations. Although co-infection events occur, the success of this approach depends on the occurrence of productive co-infection and the collection of samples for later analysis. Because the frequency and distribution of co-infection across host species and populations is unknown, designing systematic sampling strategies for detecting co-infection events would be difficult. Rather, these events are captured on an ad hoc basis. Moreover, unknowns in the route of infection, the level and time of exposure, and diversity in the host response due to existing natural or induced immunity limits the ability of this approach to reliably assess genetic compatibility of reassortant viruses. Similarly, if the viruses analyzed have disparate tissue and cell tropism or fitness *in vivo*, this approach may not accurately portray reassortment potential.

The use of replication incompetent viruses provides another alternative method for the analysis of genetic compatibility between gene segments from two influenza viruses. In these model systems, viral replication can be assessed in cell culture lines that are engineered to stably express an essential viral protein that is missing from the "replication-incompetent" virus strains used for infection. For example,

[1752] Steel J, Lowen AC (2014) Influenza A virus reassortment. *Current topics in microbiology and immunology* 385: 377-401

the replacement of the PB2 gene with a GFP-expression construct that has the necessary flanking, non-coding and packaging sequences from the viral genome can only replicate in cell lines that stably express exogenous PB2.[1753] The result is a virus that is biologically constrained to replication in that cell line. Several replication incompetent model systems have been made, and these systems have been used to assess the genetic compatibility of virus gene segments by targeted reassortment resulting in the generation of a clonal replication incompetent virus.[1754,1755,1756] For example, the genetic background of a lab-adapted strain was compatible with the HA and NA of a high pathogenicity avian H5N1 virus.[1757] However, this system has not yet been used to broadly assess reassortment potential by the identification of replication incompetent reassortant viruses from a mixed population after transfection of 16 gene segments or fewer, as is the case during co-infection. One major drawback is that this approach does not capture the complex selection pressures observed *in vivo*. Additionally, results may not translate to reassortment in humans, and findings may not be generalizable to other virus contexts.

A final alt-GoF approach utilizes *in vitro* virus-free methods to investigate genetic compatibility of viral gene segments in isolation. In particular, forward genetic screens can be used to identify novel gene segment combinations or reassortment events that contribute to a phenotype underlying viral fitness and infectivity, such as polymerase activity. Though the simplicity and relatively high-throughput nature of these methods renders them appealing as a screening approach for the evaluation of genetic compatibility between two viruses, these approaches are inherently limited to the characterization of phenotypes previously identified in other experiments. In addition, results may not be recapitulated in the context of the full virus or *in vivo*.

15.8.3.1.3 Summary – Benefits of GoF Approaches Relative to Alt-GoF Approaches

Table 15.37 summarizes the benefits and limitations of GoF and alt-GoF approaches that assess the potential for reassortment between two wild type viruses. Taken together, GoF approaches are uniquely capable of *proactively* assessing the potential for *any* two influenza viruses to reassort, as well as for comprehensively evaluating the viability of various gene combinations. Notably, the outcomes of forced laboratory reassortment events may provide limited insight into the likelihood that such reassortment events will occur in nature, as natural reassortment depends on complex factors such as the rate of co-infection and the distribution of genetically compatible viruses (which are unknown). In addition, the relevance of this information for human populations depends on the suitability of animal models. Although surveillance-based approaches can provide broad insight into of the prevalence and distribution of reassortment viruses in different host populations, their utility is severely limited by the quality and availability of surveillance data. Similarly, the analysis of humans or animal isolates during co-infection is an unreliable method for determining the reassortment potential and genetic compatibility of two viruses, and opportunities for such studies are rare. The use of replication incompetent viruses is a promising approach for assessment of the genetic compatibility and reassortment potential between two viruses, but this system is not commonly used for this purpose and requires further validation. Moreover, it cannot capture the complex selection pressures observed *in vivo* and may not translate to mechanisms of reassortment in humans. Although the use of *in vitro* virus free systems is useful from an initial screening approach, results may not be recapitulated during the complete viral life cycle.

[1753] Ozawa M *et al* (2011) Replication-incompetent influenza A viruses that stably express a foreign gene. *The Journal of general virology* 92: 2879-2888

[1754] Ibid.

[1755] Martínez-Sobrido L *et al* (2010) Hemagglutinin-Pseudotyped Green Fluorescent Protein-Expressing Influenza Viruses for the Detection of Influenza Virus Neutralizing Antibodies. *J Virol* 84: 2157-2163

[1756] Baker SF *et al* (2014) Influenza A and B virus intertypic reassortment through compatible viral packaging signals. *Journal of virology* 88: 10778-10791

[1757] Ozawa M *et al* (2011) Replication-incompetent influenza A viruses that stably express a foreign gene. *The Journal of general virology* 92: 2879-2888

Table 15.37. Summary of the Benefits of GoF Approaches For the Study of Reassortment

Scientific Knowledge Benefits— What is the Potential for Reassortment Between Two Influenza Virus Strains?		
Experimental Approach	**Benefits**	**Limitations**
GoF #1a [1]*: Targeted reassortment by combining viral gene segments from two or more virus genotypes to generate a single virus (*in vitro*)	• Determine whether two viruses *can* reassort o Can assess viability and compatibility of select/specific gene segment combinations, independent of relative fitness (of wild type versus reassortant viruses) • Proactive – can be performed using virus gene segments that have not reassorted in nature	• *Narrow breadth*: Results may not generalize to other virus strains • *Translatability*: Results from representative animal models may not translate to humans • *Artificiality*: Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature o Likelihood of multiple reassortant events is lower than with reassortment between two viruses o Approach does not capture how selection pressures and relative fitness among virus genotypes influence reassortment outcomes o Approach does not capture impact of time and dose on reassortment outcomes
GoF #1b [1]: Targeted reassortment by combining viral gene segments from two or more virus genotypes to generate a single virus (*in vivo*)		• *Narrow breadth*: Results may not generalize to other virus strains • *Translatability*: Results from representative animal models may not translate to humans • *Artificiality*: Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature o Approach does not capture how selection pressures and relative fitness among virus genotypes influence reassortment outcomes o Approach does not capture impact of time and dose on reassortment outcomes • Selection pressures *in vitro* are less complex than *in vivo*

Table 15.37. Summary of the Benefits of GoF Approaches For the Study of Reassortment

Scientific Knowledge Benefits— What is the Potential for Reassortment Between Two Influenza Virus Strains?

Experimental Approach	Benefits	Limitations

GoF #2a [2]:
Forward genetic screen by comprehensive targeted reassortment generating a panel of clonal reassortants to identify compatible virus genotypes (*in vivo*)
	• Determine whether two viruses *can* reassort
	  ○ Can assess viability and compatibility of all possible gene segment combinations, independent of relative fitness (of wild type versus reassortant viruses)
	• Proactive – can be performed using virus gene segments that have not reassorted in nature
		• Narrow breadth – Results may not generalize to other virus strains
		• Translatability – Results from representative animal models may not translate to humans
		• Artificiality – Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature
		  ○ Approach does not capture how selection pressures and relative fitness among virus genotypes influence reassortment outcomes
		  ○ Approach does not capture impact of time and dose on reassortment outcomes

GoF #2b [2]:
Forward genetic screen by comprehensive targeted reassortment generating a panel of clonal reassortants to identify compatible virus genotypes (*in vitro*)
		• Narrow breadth – Results may not generalize to other virus strains
		• Translatability – Results from cell culture models may not translate to humans
		• Artificiality – Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature
		  ○ Approach does not capture how selection pressures and relative fitness among virus genotypes influence reassortment outcomes
		  ○ Approach does not capture impact of time and dose on reassortment outcomes
		• Selection pressures *in vitro* are less complex than *in vivo*

Table 15.37. Summary of the Benefits of GoF Approaches For the Study of Reassortment

Scientific Knowledge Benefits— What is the Potential for Reassortment Between Two Influenza Virus Strains?		
Experimental Approach	**Benefits**	**Limitations**

GoF #3a [3]:
Non-targeted reassortment with up to 16 gene segments from two different viruses generating a mixed population of recombinant viruses to select for compatible virus genotypes with enhanced infectivity (*in vivo*)
	• Determine whether two viruses *can* reassort
	  ○ Can assess viability and compatibility of many gene segment combinations by controlling for disparity in fitness between reassortants and wild type viruses
	• Proactive – can be performed using virus gene segments that have not reassorted in nature
	• Gain insight into how host pressures influence reassortment outcomes and population frequency
	  ○ Can evaluate selection pressures independent from co-infection
		• Narrow breadth – Results may not generalize to other virus strains
		• Translatability – Results from representative animal models may not translate to humans
		• Artificiality – Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature
		  ○ Approach does not capture impact of time and dose on reassortment outcomes

GoF #3b [3]: Non-targeted reassortment with up to 16 gene segments from two different viruses generating a mixed population of recombinant viruses to select for compatible virus genotypes with enhanced fitness (*in vitro*)		• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from cell culture models may not translate to humans • Artificiality – Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature ○ Approach does not capture impact of time and dose on reassortment outcomes • Selection pressures *in vitro* are less complex than *in vivo*

Table 15.37. Summary of the Benefits of GoF Approaches For the Study of Reassortment

Scientific Knowledge Benefits— What is the Potential for Reassortment Between Two Influenza Virus Strains?		
Experimental Approach	**Benefits**	**Limitations**
GoF #4a [4]: Co-infection with two wild type viruses to select for compatible virus genotypes with enhanced infectivity (*in vivo*)	• Determine whether two viruses *can* reassort o Gain insight into the compatibility of virus gene segments • Proactive – can be performed using viruses that have not reassorted in nature • Gain insight into how host pressures influence reassortment outcomes and population frequency o Mimics natural scenario under which reassortment can occur	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from representative animal models may not translate to humans • Artificiality – Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature o Approach may not capture impact of time and dose on reassortment outcomes o Approach may not capture the full potential for reassortment if there are large disparities in fitness between wild type viruses and other reassortants or if there is inefficient co-infection *in vivo* o Viruses displaying distinct tissue/cell tropism *in representative model systems* are less likely to reassort
GoF #4b [4]: Co-infection with two wild type viruses to select for compatible virus genotypes with enhanced fitness (*in vitro*)	• Determine whether two viruses *can* reassort o Gain insight into the compatibility of virus gene segments • Proactive – can be performed using viruses that have not reassorted in nature • Gain insight into how host pressures influence reassortment outcomes and population frequency o Can evaluate selection pressures independent from co-infection (as this can be controlled for *in vitro*)	• Narrow breadth – Results may not generalize to other virus strains • Translatability – Results from cell culture models may not translate to humans • Artificiality – Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature o Approach does not capture impact of time and dose on reassortment outcomes o Approach may not capture the full potential for reassortment if there are large disparities in fitness between wild type viruses and other reassortants • Selection pressures *in vitro* are less complex than *in vivo*

Table 15.37. Summary of the Benefits of GoF Approaches For the Study of Reassortment		
Scientific Knowledge Benefits— What is the Potential for Reassortment Between Two Influenza Virus Strains?		
Experimental Approach	**Benefits**	**Limitations**
Alt-GoF #1 [1]: Analysis of surveillance data to determine the occurrence and prevalence of reassortment	• Determine whether reassortant viruses exist **in nature** o **Directly** translates to **human** disease when human isolates are analyzed when applicable o Analyzes broad data sets applicable to many strains o Gain insight into the prevalence and distribution of reassortant viruses across host populations	• Reactive – Involves analysis of viral isolates that already exist in nature • Translatability – Results may not translate to reassortment in humans when animal isolates are analyzed o Whether animals under study are representative models for human disease has not been established • Limited by the quality and availability of surveillance data o Incomplete genome sequences limit the identification of gene segment ancestry (i.e., reassortants) o Reassortment between genetically similar strains may not be evident or distinguishable from genetic drift o Requires large data sets for reliable phylogenetic analysis o High viral diversity, as observed in avian populations, limits the ability to distinguish between reassortment and wild type gene segments

Table 15.37. Summary of the Benefits of GoF Approaches For the Study of Reassortment

Scientific Knowledge Benefits— What is the Potential for Reassortment Between Two Influenza Virus Strains?

Experimental Approach	Benefits	Limitations
Alt-GoF #2 [2]: Analysis of viral isolates from **humans** that have been co-infected with two influenza viruses	• Determine whether reassortant viruses exist **in nature** ○ **Directly** translates to **human** disease ○ Gain insight into the genetic compatibility of virus gene segments • Gain insight into how host pressures influence reassortment outcomes and population frequency	• Reactive – Analysis of viral isolates that already exist in nature • Reassortment between genetically similar strains may not be evident or distinguishable from genetic drift • Likelihood of sample collection – Co-infection events may be rare; Viruses displaying distinct tissue/cell tropism are less likely to reassort; Isolates from patients that are infected with two viruses may not be collected, identified, or saved for further analysis • Human populations display variable immune responses due to differences in vaccination, previous exposures to influenza, and host factors complicating interpretation of selection pressures impacting reassortment
Alt-GoF #3 [3]: Analysis of viral isolates from **animals** that have been co-infected with two influenza viruses	• Determine whether reassortant viruses exist **in nature** ○ Gain insight into the genetic compatibility of virus gene segments • Gain insight into how host pressures influence reassortment outcomes and population frequency	• Reactive – Analysis of viral isolates that already exist in nature • Reassortment between genetically similar strains may not be evident or distinguishable from genetic drift • Likelihood of sample collection – Co-infection events may be rare; Viruses displaying distinct tissue/cell tropism are less likely to reassort; Isolates from animals that are infected with two viruses may not be collected, identified, or saved for further analysis • Translatability – Results may not translate to reassortment in humans ○ Whether animals under study are representative models for human disease has not been established

Table 15.37. Summary of the Benefits of GoF Approaches For the Study of Reassortment

Scientific Knowledge Benefits— What is the Potential for Reassortment Between Two Influenza Virus Strains?

Experimental Approach	Benefits	Limitations
Alt-GoF #4 [4]: *In vitro*, replication incompetent model system: Targeted reassortment by combining viral gene segments from two or more virus genotypes to generate a single virus[b]	• Gain insight into genetic compatibility of virus gene segments • Proactive – can be performed using virus gene segments that have not reassorted in nature	• Translatability – Results may not translate to reassortment in humans • Narrow breadth – Results may not generalize to other virus strains • Limited Utility – Replication incompetent systems have only been developed and validated for a limited number of strains o Use of existing models that make use of gene segments derived from lab-adapted strains will depend on genetic compatibility • Selection pressures *in vitro* are less complex than *in vivo* • Artificiality – Laboratory results may not reflect likelihood of reassortment or behavior of reassortant viruses in nature o Approach does not capture how selection pressures and relative fitness among virus genotypes influence reassortment outcomes o Approach does not capture impact of time and dose on reassortment outcomes
Alt-GoF #5 [5]: *In vitro*, virus-free: Forward genetic screen to evaluate genetic compatibility of virus gene segments for a phenotype underlying fitness	• Gain insight into the compatibility of virus gene segments • Proactive – can be performed using virus gene segments that have not reassorted in nature	• Simplicity of model system – Results based on the study of a viral protein/phenotype in isolation may not be recapitulated in the context of the full virus • Narrow breadth – Results may not generalize to other virus strains

[a] *GoF and alt-GoF approaches are listed in numerical order. Numbers in brackets reference the order in the landscape tables (Supplementary Information).*

[b] *To date, replication incompetent systems have only been used for targeted reassortment experiments, but in principle these systems could be used for non-targeted reassortment studies (i.e., transfection of cells with multiple gene segments from two or more viruses to broadly assess reassortment compatibility.)*

15.8.4 Benefits of GoF Approaches to Surveillance

The importance of reassortment in influenza virus biology is highlighted by its role in the emergence of human pandemic viruses with minimal population immunity– all four of the influenza pandemics that have occurred over the past century were likely caused by strains that arose through reassortment between influenza viruses, although the role of reassortment in the emergence of the 1918 pandemic virus is controversial.[1758,1759,1760,1761,1762] While the emergence of reassortant viruses cannot yet be predicted, surveillance for reassortant viruses to assess their occurrence and prevalence in nature is of interest for pandemic preparedness, and as such is one of the factors considered in pandemic risk assessments (discussed further below). Given the importance of epistasis in influenza biology, determining whether a reassortant virus poses an increased risk to human populations relative to its parental viruses poses a major challenge.

Analysis of the phenotypic properties of reassortant viruses in a laboratory setting, in particular fitness, pathogenicity, and transmissibility, provides insight into the properties associated with viable reassortants and can call attention to particular reassortant viruses that display phenotypic properties of concern. This information can inform evaluation of the risk posed by particular reassortant viruses detected in nature.

GoF approaches that proactively determine the reassortment potential between two viruses and phenotypic properties of reassortant viruses represent an efficient method for generating a breadth of information that can inform rapid analysis of surveillance data. However, whether laboratory results translate to the field strains of interest in nature is uncertain, given differences in the genetic sequences of the laboratory and field strains and the inherent artificiality of studies conducted in model systems in a laboratory setting. Characterization of field viruses, an alt-GoF approach, provides direct insight into the phenotypic properties of reassortant viruses of interest. However, this approach is reactive and depends on the availability of viral isolates or the publication of a high-quality, complete genome sequence for synthetic reconstruction of the virus. Additionally, this approach provides limited mechanistic insight into the relative fitness of reassortant and parental viruses, due to the high genetic diversity among circulating influenza viruses, and is subject to the same concerns about the translatability of laboratory studies in model systems as GoF approaches.

The benefit of using experimental data about reassortant viruses (both GoF and alt-GoF) to aid the interpretation of surveillance data is severely constrained by the quality and availability of existing genetic surveillance data. Reassortment events are most commonly identified through individual phylogenetic analysis of each viral gene segment to identify its origin and ancestry.[1763] This requires full genome sequences and large sequence databases for effective determination of phylogenetic ancestry, which are not always available, particularly for influenza viruses isolated from animal reservoirs. In particular, the surveillance of swine populations, thought to play an important role in the generation of reassortant viruses with pandemic potential due to their ability to be infected with both avian and human

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[1758] Morens DM, Fauci AS (2007) The 1918 influenza pandemic: insights for the 21st century. *The Journal of infectious diseases* 195: 1018-1028

[1759] Belshe RB (2005) The origins of pandemic influenza--lessons from the 1918 virus. *The New England journal of medicine* 353: 2209-2211

[1760] Worobey M *et al* (2014) Genesis and pathogenesis of the 1918 pandemic H1N1 influenza A virus. *Proc Natl Acad Sci U S A* 111: 8107-8112

[1761] Steel J, Lowen AC (2014) Influenza A virus reassortment. *Current topics in microbiology and immunology* 385: 377-401

[1762] Smith GJ *et al* (2009) Dating the emergence of pandemic influenza viruses. *Proc Natl Acad Sci U S A* 106: 11709-11712

[1763] Steel J, Lowen AC (2014) Influenza A virus reassortment. *Current topics in microbiology and immunology* 385: 377-401

strains of influenza, is lacking.[1764] One factor that contributes to the dearth of high-quality genetic data to support reassortment analyses is that current diagnostics are not equipped to rapidly screen and detect reassortants, though several methods have been proposed.[1765] Given these limitations, GoF and alt-GoF approaches to study reassortment currently provide minimal benefits to the interpretation of surveillance data. Full realization of their potential benefits will require significant expansion of genetic surveillance for reassortant viruses, particularly in swine populations, which will pose challenges due to producers' historical unwillingness to share data with the public health community.[1766]

The ability of GoF and alt-GoF approaches to inform assessment of the risk posed by reassortant viruses detected through surveillance is summarized in Table 15.38. Taken together, both GoF and alt-GoF approaches provide information about the phenotypic properties of reassortant viruses detected through surveillance, which can inform analysis of their potential risks to human populations. The proactive nature of GoF studies facilitates more rapid assessment of surveillance data, but results may not translate to the strains observed in nature. In contrast, alt-GoF approaches provide more relevant information by directly studying the surveillance strains of interest but generate information after strains have been detected and require a viral isolate or high-quality genetic data for synthetic reconstruction of the virus. However, both approaches currently provide minimal benefits to the interpretation of surveillance data due to the poor quality of genetic surveillance data for the study of reassortment. Full realization of their benefits will require significant expansion of surveillance networks, particularly in swine populations, as well as increasing the quantity of surveillance isolates that are subjected to full genome sequencing.

[1764] Vincent A *et al* (2014) Review of influenza A virus in swine worldwide: a call for increased surveillance and research. *Zoonoses and public health* 61: 4-17

[1765] Poon LL *et al* (2010) Rapid detection of reassortment of pandemic H1N1/2009 influenza virus. *Clinical chemistry* 56: 1340-1344

[1766] (2015) Swine influenza surveillance. Interview with veterinary influenza researcher.

Table 15.38. Summary of the Benefits of GoF Approaches Involving Reassortment		
Benefits to Surveillance – Inform Evaluation of the Risk Posed by Circulating Reassortant Viruses Detected Through Surveillance		
Approach	**Benefits**	**Limitations**
GoF Experimental Approaches: Determination of the reassortment potential between two viruses and the phenotypic properties of viable reassortant viruses	• Efficient generation of a breadth of information that can inform analysis of surveillance data • Proactive – generation of information prior to observation of reassortants in nature enables rapid assessment when similar reassortants emerge	• Results may not translate to field strains of interest in nature ○ Genetic differences between laboratory and field strains ○ Artificiality of laboratory experiments • Limitations in existing genetic surveillance data severely constrain the ability to detect reassortant viruses
Alt-GoF Experimental Approaches: Phenotypic characterization of wild type reassortant viruses detected through surveillance	• Provides direct insight into the phenotypic properties of reassortant viruses of interest	• Reactive – generation of information following emergence of reassortants in nature • Limited by the availability of viral isolates or the publication of high-quality, complete genome sequences for synthetic reconstruction of viruses detected through surveillance • Limitations in existing genetic surveillance data severely constrain the ability to detect reassortant viruses

15.8.5 Benefits to Decision-Making in Public Health Practice and Policy

GoF reassortment studies have potential to benefit two aspects of public health practice and policy. First, the results of reassortment studies may stimulate risk mitigation activities to limit the potential for risky co-infections to occur in nature in human and/or animal hosts (i.e., those co-infections that could give rise to reassortant viruses with risky properties). Second, reassortment studies may inform pandemic risk assessments of circulating animal influenza viruses, which guide downstream decision-making about pre-pandemic vaccine development and other pandemic preparedness initiatives. This section discusses the benefits of GoF approaches relative to alternative approaches for studying reassortment for each of these areas in turn.

15.8.5.1 GoF Benefits to Risk Mitigation Activities That Aim to Prevent the Emergence of Reassortant Viruses in Nature

Reassortant viruses arise in nature during co-infection of a host with two different viruses. Limiting the interaction between two different species can mitigate the risk of co-infection of either host with an adapted and an "exotic" strain (e.g., co-infection of a human with seasonal H1N1 and avian H7N9), which could give rise to a reassortant strain comprised of viral gene segments from strains adapted to both species.[1767] GoF approaches that proactively study the reassortment potential between two virus strains adapted for growth in different species provides insight into reassortants that are viable and that display

[1767] (2015k) Interviews with researchers at the National Wildlife Health Center (United States Geological Survey, Department of the Interior).

phenotypic properties of concern. This information can help to prioritize risk communication about measures to mitigate the chance of co-infections.[1768] For example, hunters would be encouraged to wear personal protective equipment while gutting birds in areas where avian viruses capable of reassorting with human seasonal viruses are circulating in game bird populations.[1769] Another example is providing guidance to staff and visitors at US National Parks about potentially risky interactions between people and wildlife and about clinical signs of infection in people, as National Parks provide an unusually high number of opportunities for humans and wildlife to mix.[1770] Researchers at the National Wildlife Health Center (United States Geological Survey, Department of the Interior), who are often called upon to provide this kind of "prevention" advice, emphasized that messaging must be targeted and meaningful for buy-in. Data from GoF reassortment studies provides an evidence base for messaging that may increase awareness and compliance among the target population. The results of GoF reassortment studies may also inform biosecurity practices at farms, with respect to interactions between farm workers and animals, interactions between different species of animals (e.g., poultry and swine at mixed-species farms), and interactions between agricultural animals and wildlife.

Environmental conditions that provide opportunities for co-infections with a human seasonal virus and an animal flu virus that has already caused human infections are of high concern regardless of results from laboratory reassortment studies (i.e., the phenotypic properties of viable reassortants).[1771] Thus, GoF studies that investigate the reassortment potential between human seasonal viruses and animal viruses that have not yet caused human infections are likely to have a larger impact on public health practice. For example, many influenza researchers expressed strong interest in understanding whether the highly pathogenic avian influenza H5N2 virus that caused widespread outbreaks in US domesticated poultry populations in the summer of 2015 is capable of reassorting with human seasonal viruses.[1772] As USDA experts expect the virus to return this fall, this information could impact risk communication and recommended biosafety practices for implementing control measures (e.g., culling animals, decontaminating farms, etc.).[1773] Because alternative experimental approaches for studying reassortment are reactive (i.e., limited to studying co-infections and reassortment events that have already occurred in nature), they are unlikely to be useful for informing public health practices related to reassortment prevention.

Notably, the likelihood that co-infections and subsequent reassortment occurs also depends on complex ecological factors such as the distribution of viruses within and among reservoir species, which are poorly understood. An improved understanding of these factors is needed to further refine risk communication and community-level intervention efforts that aim to prevent the emergence of novel influenza viruses in human populations through reassortment. These factors can be studied using alternative approaches such as characterizing the prevalence and distribution of influenza viruses circulating within and between animal reservoir species, determining the frequency of co-infection events in nature and the parameters determining outcomes of co-infection, and identifying relevant intermediate hosts. Together, this information can provide insight into the factors that drive reassortment events in nature.

Taken together, GoF studies that proactively study the reassortment potential between human seasonal viruses and animal viruses that have not yet caused human infections may help to prioritize risk

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[1768] (2015h) Interviews with influenza researchers.
[1769] (2015k) Interviews with researchers at the National Wildlife Health Center (United States Geological Survey, Department of the Interior).
[1770] Ibid.
[1771] Zhu Y *et al* (2013a) Human co-infection with novel avian influenza A H7N9 and influenza A H3N2 viruses in Jiangsu province, China. *Lancet* 381: 2134.
[1772] (2015h) Interviews with influenza researchers.
[1773] USDA issues plan for likely fall return of avian flu. CIDRAP. http://www.cidrap.umn.edu/news-perspective/2015/09/usda-issues-plan-likely-fall-return-avian-flu. Last Update September 21, 2015. Accessed November 7, 2015.

communication and risk mitigation measures that aim to limit cross-species interactions that would provide opportunities for co-infection. These data also provide an evidence base for risk mitigation messaging that may increase compliance among the target population. Alternative approaches can provide insight into the ecological factors that drive reassortment in nature, which is also needed to refine prioritization of risk communication and mitigation activities.

As environmental conditions that provide opportunities for co-infections with a human seasonal virus and an animal virus that has caused human infections are already of high concern, reassortment studies involving these viruses are unlikely to further increase preventive measures that are already in place.

15.8.5.2 GoF Benefits to Pandemic Risk Assessments and Downstream Decision-Making for Pandemic Preparedness

Pandemic risk assessments of circulating animal influenza viruses inform decision-making about how to invest in public health preparedness activities for influenza pandemics, particularly development of pre-pandemic vaccines. The genomic variation risk element of the Influenza Risk Assessment Tool (IRAT) used by the USG for pandemic risk assessments, described in detail in Section 15.3.5.2, includes consideration of reassortment. Specifically, reassortment between different lineages or sub-types of viruses raises the risk score for this element. GoF approaches that provide insight into the properties of reassortant viruses, in particular their fitness, transmissibility, and virulence, could be used to refine the scores associated with this risk element. In this way, GoF approaches may benefit downstream decision-making in public health policy. Because viruses that undergo risk assessments are also subjected to phenotypic characterization of virulence and transmissibility, the main benefit afforded by GoF data is that it can be generated proactively to enable evaluation of pandemic risk as soon as the genetic sequence of a virus is published.

In addition to genomic variation, several other types of information related to the properties of the virus are considered in the risk assessment: phenotypic data (i.e., transmissibility and virulence in ferrets), epidemiological data (i.e., the number and severity of human infections), and ecological data (i.e., factors related to infections in animals). In general, the genomic variation risk element is of low- to intermediate-importance relative to these other factors, though corroboration of phenotypic data adds value to the assessment by increasing certainty in downstream decisions. However, as discussed in detail in Section 15.3.5.2, this risk element may play a relatively more important role in the assessment when a novel virus first emerges in human populations, if sequences are published prior to the shipping of viral isolates to the US. The ability to evaluate risk based on genetic sequence data enables a rapid risk assessment, which may trigger the decision to develop a CVV, providing a head start on vaccine production that would be valuable in the event of a pandemic.

15.9 Evaluation of the Globalization Potential of GoF Research

15.9.1 Summary of Findings

Whether risks and benefits are equally distributed across populations is an important consideration in any risk-benefit comparison. For GoF research involving PPPs, the risks are global. This section assesses the potential for select benefits of GoF research conducted in the US to diffuse globally, in order to inform the comparison of risks and benefits associated with this research. The potential for three types of GoF benefits to globalize are considered:

- Improvements to the production of egg- and cell-based influenza vaccines,

- Assistance in the development of new influenza and coronavirus small molecule antivirals, and
- Contributions to risk assessments of circulating animal influenza viruses (pre-pandemic), which in turn inform prioritization of pandemic preparedness activities such as the development of pre-pandemic vaccines.

15.9.1.1 Improvements to the Production of Egg- and Cell-Based Influenza Vaccines

Several developing countries have the capacity to directly harness GoF research with potential to benefit the production of egg- and cell-based influenza vaccines. Specifically, non-high income countries host 18 vaccine producers spanning eight countries, representing an increase in the number of producers and vaccine-producing countries since 2010. However, the fact that eight out of 13 influenza vaccine producers that received funding from BARDA contracts allotted in 2006 are not yet marketing an influenza vaccine highlights the slow timescale for establishing new influenza vaccine production lines in developing countries. Barriers include human factors (e.g., alleged corruption leading to delays in the construction of manufacturing facilities), technical factors (e.g., contamination of vaccine), and economic factors (e.g., lack of domestic demand). Lack of demand for influenza vaccines in-country appears to be a particularly important issue facing all producers, which is compounded by a lack of knowledge about optimal vaccination strategies, with respect to vaccine composition and the timing of vaccine delivery, in tropical regions.

US vaccine donations in the event of a pandemic provide a second pathway for GoF-derived benefits to reach developing countries. The United States donated approximately 14% of the vaccines committed to the WHO during the 2009 H1N1 pandemic response, which collectively were deployed to 77 countries. However, in 2009 both vaccine donation and distribution were significantly delayed, and logistical challenges associated with vaccine distribution further reduced and/or delayed the quantity of vaccine doses that reached developing countries' populations. Although some of these shortcomings have been addressed in theory by the WHO Pandemic Influenza Preparedness Framework, the ability of the US and the WHO to provide donated vaccines in time to mitigate the effects of a high morbidity influenza pandemic in the world's developing countries remains untested.

15.9.1.2 Assistance in the Development of Novel Influenza or Coronavirus Antivirals

The ability of foreign countries to establish production lines for new antivirals depends not only on their technical and industrial capabilities but also on their ability to negotiate complex patent issues. In cases where patent protections do not apply, the actual time needed to initiate commercial production of a US-designed or commercialized antiviral appears to be in the one to five year range. Patent protections do not apply when a patent is not recognized nationally or is abrogated during a medical emergency, or where the compound can be sublicensed from the patent owner. However, several companies in developing countries rapidly activated production of influenza antivirals in less than six months in 2005–2006, when their governments were preparing for a potential H5N1 pandemic, suggesting that a general lack of demand for influenza antivirals appears to be keeping globalization in check.

The US demonstrated its willingness to donate influenza antivirals during the 2009 H1N1 pandemic. However, problems of timeliness of supply compounded issues of suboptimal use in-country. The WHO Pandemic Influenza Preparedness Framework (developed in 2011) seeks to address timeliness issues but remains untested.

15.9.1.3 Contributions to Pandemic Risk Assessments of Circulating Influenza Viruses

The demonstration that animal influenza viruses can acquire pandemic properties in a laboratory setting may galvanize preparedness efforts in developing countries where the virus is circulating in agricultural animal or wildlife populations. For example, the 2012 demonstration that H5N1 could evolve the capacity for airborne transmission between ferrets triggered some developing countries to initiate communications campaigns to raise awareness of the risks associated with H5N1 infections among the public, public health personnel, and healthcare workers, in order to bolster early detection capabilities.

Because most developing countries in which high-risk animal influenza viruses are circulating lack the ability to assess the transmissibility and virulence of viruses in ferrets, data which critically inform pandemic risk assessments, risk assessments are carried out in collaboration with WHO and laboratory members of the GISRS (including the CDC). Similar to USG risk assessments, these risk assessments incorporate information derived from GoF research, alongside epidemiologic and virologic data, and environmental factors that influence the pandemic potential of the virus.

Downstream of a pandemic risk assessment, the ability of developing countries to implement prevention and early detection measures in response to the detection of zoonotic influenza cases or outbreaks in humans and/or animals varies widely, depending on the state of public health infrastructure, the relationship between the Veterinary Services and Public Health sectors, and the resources for investing the prevention and response activities. Thailand's ability to eradicate H5N1 from their poultry production system in response to widespread outbreaks in poultry populations as well as multiple human spillover cases in 2003 – 2006 indicates that successful eradication campaigns are possible. However, the fact that Vietnam continues to experience HPAI outbreaks since the initial 2004 – 2005 outbreak in the region highlights the challenges for successfully carrying out response activities that mitigate the risk of avian influenza spillover into human populations.

Although multiple developing countries in which zoonotic avian influenza infections have been detected in human and/or bird populations within the past five years currently have the capacity to produce pre-pandemic influenza vaccines in-country, 21 do not. As WHO does not stockpile pre-pandemic vaccines, the lack of vaccine production capabilities in some at-risk countries limits the globalization potential of GoF benefits related to pandemic risk assessments.

15.9.2 Introduction

Whether risks and benefits are equally distributed across populations is an important consideration in any risk-benefit comparison. For GoF research involving PPPs, the risks– that biosafety or biosecurity incidents associated with the conduct of GoF research involving PPPs may spark a pandemic–are global. In contrast, whether GoF benefits are globally distributed is likely to vary by the type of benefit considered. The extent to which these benefits can be globalized influences whether risks and benefits are equally distributed for a particular type of GoF study.

To inform deliberations on this issue, this section evaluates the globalization potential of select GoF benefits to public health in developing countries. That is, the potential for the outputs of GoF research conducted in the US to benefit the health of human populations in low- and middle-income bracket countries, as defined by the World Bank, is assessed.[1774]

[1774] This classification system is used by the World Health Organization.
The World Bank, "Country and Lending Groups," http://data.worldbank.org/about/country-and-lending-groups. Accessed July 7, 2015.

Three types of GoF benefits are considered in this section:

- Benefits to the development and production of egg- and cell-based influenza vaccines,
- Benefits to the development of new antivirals for influenza viruses or coronaviruses, and
- Benefits to risk assessments of circulating animal influenza viruses (pre-pandemic), which may in turn stimulate pandemic preparedness activities such as enhanced surveillance and the development of pre-pandemic vaccines.

Currently, there are no FDA-approved vaccines for MERS-CoV or SARS-CoV.[1775,1776] The development of CoV vaccines is an active area of research, including research into multiple vaccine platforms (e.g., recombinant vaccines, live attenuated vaccines, DNA vaccines, etc.). GoF research that alters host tropism and enhances virulence in appropriate animal models has the potential to benefit the development of CoV vaccines, through the generation of mouse-adapted viruses that serve as a robust animal model for testing the safety and efficacy of vaccine candidates. However, which type of vaccine will be most rapidly developed and will prove to be most effective is not clear based on current CoV vaccinology research. Because the resources and expertise that are required to develop production capacity for different types of vaccines varies, the globalization potential and barriers to globalization for hypothetical CoV vaccines cannot be evaluated. Similarly, uncertainty in factors related to the globalization of benefits related to the development of novel influenza vaccines (derived from GoF approaches that lead to evasion of existing natural or induced adaptive immunity or that enhance virulence) precludes a meaningful evaluation of the globalization of these benefits. Thus, the scope of this assessment of the globalization potential of benefits related to vaccines is limited to GoF benefits to the development and production of existing influenza vaccines.

The globalization potential for benefits to the development of therapeutics is evaluated based on case studies on the globalization of production and use of the four influenza antivirals that are currently licensed in developed countries, which are all small molecule compounds. Therapeutics targeting MERS-CoV and SARS-CoV are currently in the development phase and include small molecule compounds as well as other types of therapeutics (e.g., monoclonal antibodies).[1777] Setting up hypothetical future production lines for CoV small molecule antiviral drugs is likely to require a similar level of resources and expertise as needed for the development of production lines for influenza small molecule drugs. As such, and in contrast to the evaluation of benefits to vaccine production, this evaluation of the globalization potential of benefits to therapeutic development applies to relevant research involving CoVs as well as research involving influenza viruses.

Currently, GoF approaches involving coronaviruses do not have the potential to benefit surveillance. Although CoV researchers stated that they could envision using information about the molecular determinants of human adaptation and virulence to assess the risk posed by animal CoVs circulating in nature, similar to the influenza field, this application is currently unfeasible for two reasons: (1) CoV

[1775] Centers for Disease Control and Prevention (CDC), "Middle East Respiratory Syndrome (MERS)," June 2, 2015, http://www.cdc.gov/coronavirus/mers/about/prevention.html. Accessed July 7, 2015.

[1776] World Health Organization, "Severe Acute Respiratory Syndrome (SARS)," December 1, 2013, http://www.who.int/immunization/topics/sars/en/. Accessed July 7, 2015.

[1777] During the 2003 SARS-CoV epidemic, Ribavirin was used; however, it "did not appear to have a significant effect," and a study of patients treated with Ribavirin indicated "that ribavirin provided no benefit in the resolution of symptoms or survival." In: Els Keyaerts, Leen Vijgen, Marc Van Ranst, "Current Status of Antiviral Severe Acute Respiratory Syndrome Coronavirus Research," *Coronaviruses: Molecular and Cellular Biology*, ed. Volker Thiel (Norfolk: Caister Academic Press, 2007), p. 328.

surveillance networks are extremely limited, with large gaps in coverage in humans and animals and (2) The state of knowledge about the molecular determinants of human adaptation and virulence is poor.[1778] Therefore, the analysis of the globalization potential of benefits related to surveillance and pandemic risk assessments is restricted to research involving influenza viruses.

This assessment evaluates each benefit (i.e., benefits to influenza vaccine production, benefits to the development of influenza, and benefits to risk assessments of zoonotic influenza viruses) in turn. GoF benefits to vaccines and therapeutics may globalize in two ways:

- Research results can be applied by third countries, with or without US assistance, to the development and production of vaccines and antivirals abroad.
- Research results can be applied to the development and production of vaccines and antivirals in the US, to be relinquished for distribution to third countries through the World Health Organization (WHO) in the event of a pandemic or through non-pandemic assistance programs.

To evaluate the globalization potential of GoF benefits to vaccines and therapeutics, indigenous capabilities for vaccine and therapeutic production are first described in order to assess the ability of developing countries to harness the outputs of GoF research directly. Second, relevant United States and WHO international assistance doctrines and frameworks are described, and examples of prior US assistance are reviewed. Taken together, these two parts enable a qualitative assessment of the degree to which GoF benefits to PPP vaccines and therapeutics may diffuse globally, as well as the timescale over which those benefits are expected to internationalize. To evaluate the globalization potential of GoF benefits to pandemic risk assessments of animal influenza viruses, this section reviews whether and GoF research contributes to risk assessments conducted in developing countries in which high-risk animal influenza viruses are circulating as well as the ability of countries to mount responsive pandemic preparedness activities.

15.9.3 Potential Benefit 1- Improvements in the Design and Production of Vaccines

Several types of GoF research have potential to improve the development and production of egg- and cell-based influenza vaccines, namely GoF research that enhances virus production that leads to evasion of therapeutics, that enhances pathogenicity, and that leads to evasion of existing natural or induced adaptive immunity. Here the benefits of GoF research to influenza vaccine production are briefly summarized. For a detailed analysis of each GoF benefit, refer to the individual entries in the section devoted to the benefits of each GoF phenotype above.

GoF research that enhances virus production leads to the generation of higher-yield vaccine backbone strains and candidate vaccine viruses (CVVs) as well as the identification of genetic markers that enhance the growth of vaccine viruses. These outputs can benefit vaccine production in two ways: (1) through the direct use of higher yield vaccine viruses by CVV developers and (2) through the incorporation of high-yield markers into existing vaccine viruses by CVV developers or manufacturers. Use of higher-yield vaccine viruses shortens vaccine production timelines by increasing the rate of bulk antigen production, which improves the availability and efficacy of influenza vaccines. Specifically, streamlined vaccine production processes will translate to faster availability of vaccines during a pandemic and will enable selection of seasonal strains closer to the start of flu season, reducing the likelihood of vaccine mismatch. Increasing the yield of vaccine antigen per egg or cell also reduces the manufacturing cost of the vaccine, which may translate to a lower cost per vaccine dose.

[1778] (2015b) Interviews with coronavirus researchers.

GoF research that enhances pathogenicity may lead to the identification of molecular markers of enhanced pathogenicity, and GoF research that leads to evasion of therapeutics may lead to the identification of molecular markers of antiviral resistance. Once validated across many strain contexts, these markers may be removed from the HA and NA genes of vaccine strains through targeted mutagenesis, thereby increasing the safety of the vaccine production process.

GoF research that leads to the evasion of existing natural or induced immunity may lead to the identification of molecular markers for antigenic change and provides insight into the evolutionary mechanisms driving antigenic drift in nature. This information has potential to improve the strain selection process in several ways, all of which increase the likelihood that vaccine strains will match circulating strains during their target flu season. Ultimately, better vaccine match translates to improved vaccine efficacy, which will mitigate the public health impacts of seasonal influenza epidemics.

For all types of GoF research, these benefits may be harnessed by developing countries through direct application of GoF research outputs to indigenous influenza production lines or may benefit developing countries indirectly through US seasonal and pandemic vaccine donations.

15.9.3.1 Capacity for Direct Application of GoF Research Outputs to Foreign Influenza Vaccine Production

As summarized above, GoF research has potential to benefit the development and production of influenza vaccines through modifications to vaccine strains used for the production of egg- and cell-based vaccines, which could enhance the safety of the vaccine production process and could improve the quality and availability of vaccines. High yield CVVs for seasonal and pandemic influenza strains, which serve as the basis for vaccine strains used for large-scale manufacturing of vaccines, are developed by WHO Collaborating Centres (WHOCCs) for Influenza and other collaborating laboratories.[1779,1780,1781] The WHO Pandemic Influenza Preparedness Framework stipulates that influenza CVVs be made available from WHOCCs to any influenza vaccine manufacturer and any other laboratory who makes a request, as long as the requestor meets appropriate biosafety requirements to receive the strain in question.[1782] The GISRS provides the international framework for the sharing of such biological materials between laboratories around the world.[1783] Vaccine manufacturers then serially passage CVVs to adapt the viruses for growth in their production systems and further enhance yields, in order to develop vaccine seed strains that are used for large-scale production of vaccines. Thus, any benefits to strain selection of seasonal influenza viruses (i.e., GoF research that leads to evasion of existing natural or induced adaptive immunity), which determine the composition of CVVs, are inherently global. GoF research that leads to the identification of molecular markers of high-yield, virulence, or antiviral resistance (i.e., GoF research that enhances virus production, enhances virulence, or leads to evasion of therapeutics) can be applied by CVV developers or vaccine manufacturers. That is, molecular markers of high growth can be incorporated in, or molecular markers of antiviral resistance or virulence can be mutated out, of CVVs or vaccine seed strains by CVV developers or manufacturers, respectively.

[1779] World Health Organization (WHO), "Influenza: Influenza vaccine viruses and reagents," http://www.who.int/influenza/vaccines/virus/en/. Accessed July 7, 2015.

[1780] World Health Organization (WHO), "Influenza: Virus Sharing," http://www.who.int/influenza/pip/virus_sharing/en/. Accessed July 7, 2015.

[1781] World Health Organization (WHO), *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits* (Geneva: World Health Organization Press, 2011), p. 16-17, http://apps.who.int/iris/bitstream/10665/44796/1/9789241503082_eng.pdf. Accessed July 7, 2015.

[1782] Ibid, p. 12, 15-17.

[1783] World Health Organization (WHO), "Global Health Observatory (GHO) data: Global influenza virological surveillance," http://www.who.int/gho/epidemic_diseases/influenza/virological_surveillance/en/. Accessed July 7, 2015.

Therefore, the ability of developing countries to directly benefit from GoF research conducted in the US depends on their industrial capacity to produce influenza vaccines. If a developing country has an existing commercial vaccine production line, the country could either harness GoF benefits through utilization of modified CVVs, provided by WHOCCs, or through the application of GoF research findings to vaccine seed strains developed by indigenous manufacturers. Altogether, the likelihood and timescale over which GoF benefits to vaccine production can be realized depends on two factors: (1) for those countries that do not yet have influenza vaccine production capabilities, the resources needed for and challenges associated with the establishment of new influenza vaccine production lines and (2) for those countries that already have influenza vaccine production capabilities, the country's regulatory policies governing changes in vaccine strains. Although an assessment of country-specific regulatory policies as they pertain to the use of modified vaccine strains is outside the scope of the current study, the FDA does not require regulatory approval for the commercial use of modified vaccine strains (i.e., there is no regulatory barrier for GoF benefits to vaccine production in the US).

15.9.3.1.1 Production of Influenza Vaccines Abroad

Global influenza production capacity was most recently comprehensively surveyed in 2010 by the WHO. The WHO study identified 28 manufacturers that either produced influenza vaccine or were slated to produce influenza vaccine by 2015.[1784] Each manufacturer or potential future manufacturer was then classified by the World Bank income groups of their home country (simplified to high-, medium-, or low-income).[1785] Of these, 14 manufacturers were in high-income and 14 were in middle-income countries.[1786] Based on the reported findings, there were at least eleven vaccines on the market from manufacturers in middle-income countries in 2010, with at least another eight vaccines in development.[1787] For comparative purposes, manufacturers based in high-income countries had at least 16 vaccines on the market at the time, and at least 35 additional vaccines being developed (most using novel technologies).[1788]

Table 15.39. Summary of Influenza Vaccine Production Capabilities by Country Type, 2010, WHO data[1789]

Production method	Number of producers in high-income countries*		Number of Producers in low-income countries*	
	Current	In development	Current	In development
Egg-based production**	At least thirteen	None	At least ten	At least eight
Cell-based production**	Three	At least five	One	None
Other production methods***	None	At least 30	None	None

[1784] [WHO] Technical Studies Under Resolution WHA63.1, Final Document, A/PIP/OEWG/3/2, April 4, 2011, p. 22-26. http://apps.who.int/gb/pip/pdf_files/OEWG3/A_PIP_OEWG_3_2-en.pdf. Accessed July 28, 2015.
[1785] Ibid.
[1786] Ibid.
[1787] Ibid.
[1788] Ibid.
[1789] Ibid.

Table 15.39. Summary of Influenza Vaccine Production Capabilities by Country Type, 2010, WHO data[1789]

Production method	Number of producers in high-income countries*		Number of Producers in low-income countries*	
	Current	In development	Current	In development

**Some manufacturers produce more than one type of vaccine. As a result, the sum of the number of manufacturers listed in the table is greater than the total number of manufacturers reported above.*

***Includes producers of inactivated and live attenuated vaccines.*

****Namely: recombinant haemagglutinin and viral-like particle vaccines (mammalian, insect cells, plant-based, or other), "universal" vaccines, viral vector vaccines, and DNA vaccines.*

The survey identified the following five middle-income countries as having domestic influenza vaccines: China, India, Thailand, Indonesia, and Romania.[1790,1791] Planned production lines were identified in the following nine middle-income countries: Brazil, Egypt, Kazakhstan, Mexico, Serbia, South Africa, Thailand, Iran, and Vietnam.[1792]

No updated list of active human influenza vaccine manufacturers in 2014 or 2015 has been made publicly available. A dataset of influenza producers was therefore compiled to compare the current influenza production situation with that surveyed in 2010. First, we determined which of the 28 companies identified by the WHO 2010 survey are still currently commercially producing influenza vaccines.[1793] This list was supplemented with current or planned influenza manufacturers outside of high-income countries listed in the Developing Countries Vaccine Manufacturers Network (DCVMN) directories from 2014 and 2015,[1794,1795,1796] in the International Federation of Pharmaceutical Manufacturers & Associations' Influenza Vaccine Supply Members list,[1797] and in the US Department of Health and Human Services' Influenza Vaccine International Capacity Building Portfolio.[1798] These were subsequently bolstered by searches on potential manufacturers flagged in the literature or in news

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1790 Marie-Paule Kieny, "Overview of Global and Regional Influenza Vaccine Production Capacity," presentation given at the WHO GAP-II Vaccine Production Capacity conference, Geneva, Switzerland, July 13, 2011, p.6, http://www.who.int/influenza_vaccines_plan/resources/mpk_b.pdf. Accessed October 29, 2015.

1791 Jeffrey Partridge, Marie Paule Kieny, "Global production capacity of seasonal influenza vaccine in 2011," *Vaccine* 31, no. 5 (January 2013): p. 728-731, http://www.sciencedirect.com/science/article/pii/S0264410X12015861. Accessed October 1, 2015.

1792 Ibid.

1793 The list of company names provided in note 4 was used. Jeffrey Partridge, Marie Paule Kieny, "Global production capacity of seasonal influenza vaccine in 2011," *Vaccine* 31, no. 5 (January 2013): p. 728-731, http://www.sciencedirect.com/science/article/pii/S0264410X12015861. Accessed October 1, 2015.

1794 DCVMN is a coordinating platform for vaccine producers in the developing world. Note that certain DCVMN producers are in countries that are currently classed by the World Bank as being High-Income countries (such as South Korea). For a description of the DCVMN, see: Sonia Pagliusi et al., "Developing Countries Vaccine Manufacturers Network: Doing good by making high-quality vaccines affordable for all," *Vaccine* 31 supplement 2 (April 2013): p. B176-B183, http://www.sciencedirect.com/science/article/pii/S0264410X1201701X. Accessed July 13, 2015.

1795 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p.1-96, http://www.dcvmn.org/IMG/pdf/directory.pdf. Accessed November 15, 2015.

1796 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2014," 2014, p.1-82, http://www.dcvmn.org/IMG/pdf/dcvmn_directory_2014.pdf. Accessed July 7, 2015.

1797 International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), "IFPMA Influenza task force – IVS Membership," http://www.ifpma.org/resources/influenza-vaccines/ifpma-influenza-task-force/ivs-membership.html Accessed July 7, 2015.

1798 U.S. Department of Health & Human Services, "International Influenza Vaccine Capacity Building Portfolio," https://www.medicalcountermeasures.gov/projectmaps/Who.aspx. Accessed October 1, 2015.

reports.[1799] A referenced list is provided in Section 15.9.6, and the findings are summarized in the figure below.

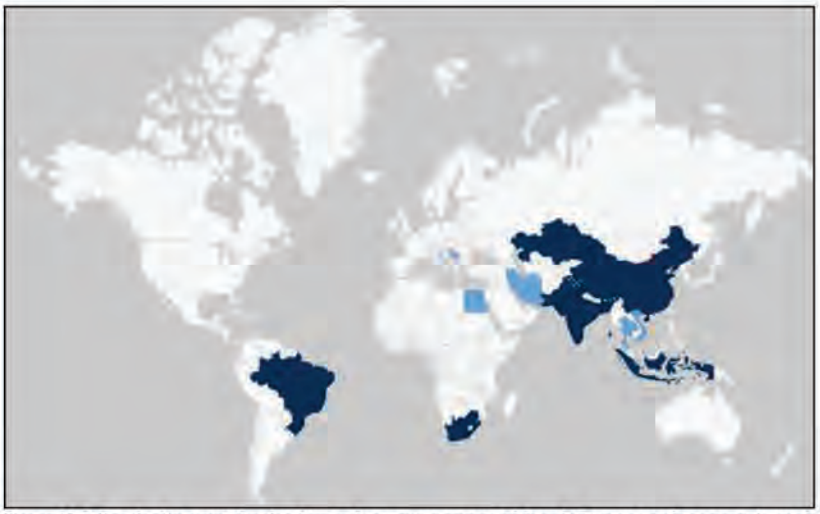

Figure 15.3. Developing countries that host at least one company with an influenza vaccine currently on the market are shaded in deep blue. Developing countries that host at least one company with R&D efforts for the production of an influenza vaccine are shaded in light blue.

Analysis of the assembled dataset reveals that the number of active producers outside of high-income countries has increased since 2010. In total, 18 companies in middle-income countries were found to be actively producing influenza vaccines, compared to eleven manufacturers in 2010.[1800] These were mostly located in China (8) and India (4), although Bangladesh, Brazil, Indonesia, Iran, Kazakhstan, and Pakistan each had one producer. One country has stopped production, since the sole Romanian manufacturer marked as active in 2010 has stopped marketing influenza vaccines.[1801] At least 13 additional companies have R&D work for influenza vaccines at various stages of completion. These companies were located in China (4), Egypt (1), Iran (2), Serbia (1), Thailand (3), and Vietnam (2).

As many of the new influenza vaccine manufacturers between 2010 and 2015 are located in countries that already had influenza vaccine production capabilities, overall the geographic distribution of production capacities outside of high-income countries has only moderately expanded. Eight countries now produce

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[1799] Jan Hendriks, Yan Liang, Bing Zeng, "China's emerging vaccine industry," *Human Vaccines* 6, no. 7 (2010): p. 602-607, http://www.tandfonline.com/doi/pdf/10.4161/hv.6.7.11933. Accessed October 29, 2015

[1800] This count excludes companies based in Taiwan, as the World Bank classes "Taiwan, China" as a "high-income" economy, separately from "China," which it classes as an "upper-middle-income" economy. See:
The World Bank, "Country and Lending Groups," http://data.worldbank.org/about/country-and-lending-groups. Accessed July 7, 2015.

[1801] "Institutul Cantacuzino nu face vaccin antigripal nici in sezonul 2015 - 2016, desi are autorizatii" [Cantacuzino Institute will not make flu vaccine in the 2015-2016 season, despite having licenses], *Ziare*, May 21, 2015, http://www.ziare.com/social/spital/institutul-cantacuzino-nu-face-vaccin-antigripal-nici-in-sezonul-2015-2016-desi-are-autorizatii-1364363. Accessed October 1, 2015.

influenza vaccines (up from five). Based on current R&D efforts, an additional five countries may become influenza vaccine producers in the future.[1802]

A lack of end-user demand appears to be a recurring and common problem that is preventing several of the middle-income firms mentioned in this section from initiating or maintaining influenza vaccine production. With respect to pandemic influenza vaccines, this issue stems from a lack of government support to purchase vaccines for pandemic preparedness purposes. For example, representatives of the Serum Institute of India argued that the Indian government's decision not to purchase an H1N1 vaccine it initially financially supported had "threatened the sustainability of influenza production capacity in India" and resulted in six million doses of unsold vaccine.[1803] With respect to seasonal influenza vaccines, this issue involves a lack of demand by individuals. For example, a presentation by a senior advisor on disease control from the Ministry of Public Health of Thailand on vaccine production plans in-country noted that there simply had been no demand for seasonal influenza vaccine before the 2004 H5N1 outbreak affected the country.[1804] Notably, the Chinese market experience has demonstrated that domestic demand for seasonal influenza vaccine increases with the income level of individuals, thus low domestic demand is to be expected outside of high income countries.[1805] This demand issue is compounded by the fact that current recommendations for the strain composition of seasonal influenza vaccines are geared toward countries in the Northern and Southern hemispheres with well-defined flu seasons, such as the United States and Australia.[1806] In contrast, well-defined seasonality does not always occur in tropical regions of the world; instead, low levels of influenza virus circulate throughout the year. In these regions, optimal vaccination strategies, including whether Northern or Southern hemisphere vaccines are more protective and when during the year vaccines are best deployed, are not well understood. Research to better understand patterns of influenza transmission and seasonality in the tropics, as well as how best to mitigate the public health burden associated with influenza through vaccination, is ongoing. This research provides an important foundation for developing countries' efforts to bolster their vaccine production capabilities and increase in-country demand in the future.

15.9.3.1.2 US Vaccine Production Assistance

Several US programs seek to support the aforementioned ability of developing countries to produce vaccines. Since seasonal vaccine production lines are adapted to produce pandemic vaccines, these pandemic preparedness programs complement seasonal influenza production assistance, and vice versa.[1807]

The US HHS supports production capabilities abroad for seasonal and pandemic influenza vaccine through funding provided by its Biomedical Advance Research and Development Authority (BARDA)

1802 Namely: Egypt, Iran, Serbia, Thailand, and Vietnam.

1803 World Health Organization (WHO), "Report of the Sixth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers," p.15, http://apps.who.int/iris/bitstream/10665/85515/1/9789241505994_eng.pdf. Accessed August 3, 2015.

1804 Suwit Wibulpolprasert, "GAP and Flu Vaccine Production in Thailand – from Public Health Policy Development to Vaccine Production," presentation given at the Second WHO Consultation on the Global Action Plan for Influenza Vaccine (GAP-II), Geneva, Switzerland, July 12, 2010, p.6, http://www.who.int/influenza_vaccines_plan/resources/suwit.pdf. Accessed October 1, 2010.

1805 Eliza Yibing Zhou, "Vaccine Development in China," *BioPharm International* 20, no. 4 (April 2007): p.1, http://www.biopharminternational.com/china-today-vaccine-development-china. Accessed October 29, 2015.

1806 Ampofo WK *et al* (2015) Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. *Vaccine* 33: 4368–4382

1807 For U.S. context, see: Executive Office of the President, President's Council of Advisors on Science and Technology, [U.S.A.] "Report to the President on Reengineering the Influenza Vaccine Production Enterprise to Meet the Challenges of Pandemic Influenza," August 2010, https://www.whitehouse.gov/sites/default/files/microsites/ostp/PCAST-Influenza-Vaccinology-Report.pdf. Accessed July 7, 2015.

branch.[1808] For example, BARDA provided funding to enable Vietnam-based VABIOTECH's planned cell-based influenza vaccine production capacity.[1809,1810] Overall, BARDA has provided financial support to 13 companies in 12 medium-income countries seeking to develop influenza vaccine production lines.[1811,1812] According to the US HHS Public Health Emergency database on BARDA's portfolio, the contracts that provided funding to these firms were all awarded in September 2006.[1813]

As of mid-2014, BARDA had provided "more than $50 million in cooperation with WHO" distributed in the form of grants to potential vaccine producers in developing countries to assist them in setting up influenza vaccine production lines.[1814] BARDA further provided "over $20 million to support vaccine adjuvant technology transfer, biomanufacturing workforce training, and clinical trial and manufacturing technical support to developing country influenza vaccine manufacturers."[1815]

Of the 13 companies that received support from BARDA, six appear to remain in the R&D phase, one has ceased production of vaccines, one appears to have halted R&D efforts, and five currently produce influenza vaccines. The status, future plans, and reasons for production delays are summarized in the following table.

Table 15.40. Summary of the Status of Influenza Vaccine Production Companies in Developing Countries That Received Funding from BARDA in 2006.

Company	Country	Status of Vaccine Production	Future Plans	Reasons for Production Delays
Acera de Birmex[1816]	Mexico	Production facility was under construction, but Birmex has stopped listing an influenza vaccine under its DCVMN 2015 product R&D description[1817]	Unknown	Unknown

1808 PATH, "PATH's Work in Vaccine Development: Low-cost influenza vaccine production," http://sites.path.org/vaccinedevelopment/influenza/vaccine-production-in-the-developing-world/. Accessed August 3, 2015.

1809 World Health Organization (WHO), "Report of the Sixth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers," Dubai, United Arab Emirates, March 18-19, 2013, p. 8, http://who.int/iris/bitstream/10665/85515/1/9789241505994_eng.pdf. Accessed August 3, 2015.

1810 Centers for Disease Control and Prevention (CDC), "Influenza Division International Activities, Fiscal Years 2012 & 2013 Annual Report," p. 121, http://www.cdc.gov/flu/pdf/international/program/2012-2013-intl-program-report.pdf. Accessed August 3, 2015.

1811 These companies are: Acera de Birmex (Mexico), BCHT (China), BioFarma (Indonesia), Cantacuzino Institute (Romania), GPO (Thailand), Instituto Butantan (Brazil), IVAC (Vietnam), RIBSP (Kazakhstan), Serum Institute of India (India), The BioVac Institute (South Africa), Torlak Institute (Serbia), VABIOTECH (Vietnam), and VACSERA (Egypt).

1812 U.S. Department of Health & Human Services, "International Influenza Vaccine Capacity Building Portfolio."

1813 Ibid.

1814 United States of America, "Report on USA implementation of Article X of the Biological and Toxin Weapons Convention," Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, Meeting of Experts, Geneva, Switzerland, August 4-8, 2014, BWC/MSP/2014/MX/INF.5, p 4 para. 10. Accessed July 7, 2015.

1815 Ibid.

1816 Luis Guillermo F. Ibarra PL et al., "Influenza Vaccine Project at Birmex," poster presented at the Eighth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers, Sao Paulo, Brazil, March 17-18, 2015, http://www.who.int/phi/8thPartnersMtg2015_Birmex_poster.pdf. Accessed November 5, 2015.

1817 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 47-48, http://www.dcvmn.org/IMG/pdf/directory.pdf. Accessed November 15, 2015.

Table 15.40. Summary of the Status of Influenza Vaccine Production Companies in Developing Countries That Received Funding from BARDA in 2006.

Company	Country	Status of Vaccine Production	Future Plans	Reasons for Production Delays
BCHT[1818]	China	Production facility construction is complete; vaccine R&D is ongoing	Begin clinical trials in 2015	N/A
BioVac Institute[1819]	South Africa	R&D for fill-finish operations (final stage of production) was ongoing in March 2015; as of November 2015 the company lists an influenza vaccine under its DCVMN 2015 marketed products[1820]	Expect to obtain a license for domestically *filling* seasonal vaccine in 2016[1821]	N/A

[1818] Jinchang Wu, "Changchun BCHT Biotechnology Co., China," poster presented at the Eighth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers, Sao Paulo, Brazil, March 17-18, 2015, http://www.who.int/phi/8thPartnersMtg2015_BCHT_poster.pdf. Accessed November 5, 2015.

[1819] Patrick Tippoo, Simphiwe Ntombela, "The BIOVAC Institute: Establishing Influenza Vaccine Manufacturing Capacity in Africa," poster presented at the Eighth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers, Sao Paulo, Brazil, March 17-18, 2015, http://www.who.int/phi/8thPartnersMtg2015_BIOVAC_poster.pdf. Accessed November 5, 2015.

[1820] Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 75-76, http://www.dcvmn.org/IMG/pdf/directory.pdf. Accessed November 15, 2015.

[1821] Patrick Tippoo, Simphiwe Ntombela, "The BIOVAC Institute: Establishing Influenza Vaccine Manufacturing Capacity in Africa," poster presented at the Eighth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers, Sao Paulo, Brazil, March 17-18, 2015, http://www.who.int/phi/8thPartnersMtg2015_BIOVAC_poster.pdf. Accessed November 5, 2015.

Table 15.40. Summary of the Status of Influenza Vaccine Production Companies in Developing Countries That Received Funding from BARDA in 2006.

Company	Country	Status of Vaccine Production	Future Plans	Reasons for Production Delays
GPO[1822,1823,1824]	Thailand	Obtained EUA for H1N1 vaccine in 2011; construction of industrial production plant ongoing	Unknown	Industrial plant construction started in 2009; corruption investigations led to suspension of funds ; construction re-approved in 2014[1825,1826,1827,1828,1829]
Cantacuzino Institute[1830]	Romania	Ceased active production of influenza vaccines for the 2015 – 2016 season[1831]	Unknown	Withdrew vaccines from the market due to low antigen concentration in 2012 and due to endotoxin contamination in 2013[1832]
IVAC[1833]	Vietnam	Conducted a Phase I clinical trial for their A/H5N1 vaccine in 2014 – 2015.[1834]	Unknown	N/A
Torlak[1835,1836]	Serbia	Preclinical trials for seasonal flu vaccine are ongoing	Unknown	N/A

1822 Somchaiya Surichan et al., "Development of influenza vaccine production capacity by the Government Pharmaceutical Organization of Thailand: Addressing the threat of an influenza pandemic," *Vaccine* 29 Supplement 1 (July 2011), p. A29-A33.

1823 Government Pharmaceutical Organization, "Our Products," http://www.intergpomed.com/Default.aspx?tabid=61. Accessed November 5, 2015.

1824 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2014," 2014, p.75-76, http://www.dcvmn.org/IMG/pdf/dcvmn_directory_2014.pdf. Accessed July 7, 2015.

1825 "Vaccine factory to restart construction," *Bangkok Post*, December 11, 2014, http://www.bangkokpost.com/lite/news/448902/vaccine-factory-to-restart-construction. Accessed November 5, 2015.

1826 Eric Palmer, "Thailand government vaccine plant at center of probe," *Fierce Pharma Manufacturing*, April 25, 2013, http://www.fiercepharmamanufacturing.com/story/thailand-government-vaccine-plant-center-probe/2013-04-25. Accessed November 5, 2015.

1827 Pongphon Samsamak, "Flu Vaccine Plant Saraburi: DSI Agrees to Look Into Irregularities," *The Nation*, April 12, 2013, retrieved at: http://www.thaivisa.com/forum/topic/632500-flu-vaccine-plant-saraburi-d-s-i-agrees-to-look-into-irregularities/. Accessed November 5, 2015.

1828 Puangchompoo Prasert, Piyanut Thammakasetchai, "Paracetamol Scandal: Action Sought against top GPO official," *The Nation*, May 2, 2013, retrieved at http://www.thaivisa.com/forum/topic/636661-paracetamol-scandal-action-sought-against-top-thai-official/. Accessed November 5, 2015.

1829 Suriyan Panyawai, "GPO chief's axing 'not political': Board's probe was thorough, chairman says," *Thailand Online News*, May 20, 2013, http://onlinenewsthailand.com/2013/05/20/gpo-chiefs-axing-not-political/. Accessed November 5, 2015.

1830 "Institutul Cantacuzino nu face vaccin antigripal nici in sezonul 2015 - 2016, desi are autorizatii," [Cantacuzino Institute will not make flu vaccine in the 2015-2016 season, despite having licenses], *Ziare*, May 21, 2015, http://www.ziare.com/social/spital/institutul-cantacuzino-nu-face-vaccin-antigripal-nici-in-sezonul-2015-2016-desi-are-autorizatii-1364363. Accessed October 1, 2015.

1831 Ibid.

1832 Ibid.

1833 "Influenza A/H5N1 Vaccine Clinical Trial (IVACFLU-A/H5N1) - Phase 1," *ClinicalTrials.gov*, October 15, 2015, https://clinicaltrials.gov/ct2/show/record/NCT02171819. Accessed October 29, 2015.

1834 Ibid.

1835 Torlak, "History," http://www.torlakinstitut.com/en/page/23/History. Accessed October 29, 2015.

1836 Torlak, "Research & Development," http://www.torlakinstitut.com/en/page/14/Research+&+Development. Accessed October 29, 2015.

Table 15.40. Summary of the Status of Influenza Vaccine Production Companies in Developing Countries That Received Funding from BARDA in 2006.

Company	Country	Status of Vaccine Production	Future Plans	Reasons for Production Delays
VABIOTECH[1837,1838]	Vietnam	Completed Phase III clinical trials for their cell-based A/H5N1 vaccine in 2012	Filed for a license in 2013	Lack of market demand for pandemic vaccine (reliance on government purchases discourages private investors)
Bio Farma[1839]	Indonesia	Fill-finished first batches in 2008; obtained licensing for the product in 2009 (first licensed product resulting from WHO tech transfer program)[1840]	N/A	N/A
Instituto Butantan[1841]	Brazil	First domestic batch produced in 2011; obtained certificate of good production practices from national regulator in 2012[1842]	N/A	N/A
Research Institute for Biological Safety Problems (RIBSP)[1843]	Kazakhstan	Pre-pandemic H5N1 and H1N1 vaccines registered with the government in 2013.[1844,1845]	N/A	N/A

[1837] VABIOTECH, "Products – Vaccine," http://www.en.vabiotech.com.vn/index.php?option=com_content&view=article&id=88&Itemid=109&lang=en. Accessed October 29, 2015.

[1838] Juliet Bryant, "Influenza vaccine manufacturing in Viet Nam: Report on the APACI Satellite session," *One Health*, 2015, http://onehealth.org.vn/influenza-vaccine-manufacturing-in-viet-namreport-on-the-apaci-satellite-session.new. Accessed October 29, 2015.

[1839] Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2014," 2014, p. 1-82, http://www.dcvmn.org/IMG/pdf/dcvmn_directory_2014.pdf. Accessed July 7, 2015.

[1840] Mahendra Suhardono, Dori Ugiyadi, Ida Nurnaeni, Imelda Emelia, "Establishment of pandemic influenza vaccine production capacity at Bio Farma, Indonesia," *Vaccine* 39, supplement 1 (July 2011): p. A22-A25, <http://www.sciencedirect.com/science/article/pii/S026441X1100689X>.

[1841] Butantan Institute, "Butantan Institute Influenza Vaccine Production," poster presented at the Eighth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers, Sao Paulo, Brazil, March 17-18, 2015, <http://www.who.int/phi/8thPartnersMtg2015_Butantan_poster.pdf>

[1842] Marcelo De Franco, Jorge Kalil, "The Butantan Institute: History and Future Perspectives," *PLoS Neglected Tropical Diseases* 8, no. 7 (July 2014): p. e2862, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4080994/pdf/pntd.0002862.pdf>

[1843] Research Institute for Biological Safety Problems (RIBSP), "Technology transfer project for Influenza Vaccine-2011/14 phase," poster presented at the Eighth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers, Sao Paulo, Brazil, March 17-18, 2015, <http://www.who.int/phi/8thPartnersMtg2015_RIBSP_poster.pdf>.

[1844] Ibid.

[1845] "Реестр отечественных поставщиков товаров фармацевтической и медицинской промышленности" [Register of domestic suppliers of goods Pharmaceutical and Medical Industries], March 13, 2015, <http://arkalyk.kostanay.gov.kz/uploads/files/1d03853ecd302518be6a42de19ca184a.doc>.

Table 15.40. Summary of the Status of Influenza Vaccine Production Companies in Developing Countries That Received Funding from BARDA in 2006.

Company	Country	Status of Vaccine Production	Future Plans	Reasons for Production Delays
Serum Institute of India Ltd.[1846]	India	Pandemic H1N1 vaccine licensed July 2010; subsequently created a trivalent seasonal influenza vaccine currently on the market.[1847, 1848]	N/A	N/A

In conclusion, more than eight years after BARDA began its assistance program, roughly two thirds of the funding recipients appear to lack an influenza vaccine product on the market. Since the method by which the funding recipients were picked has not been made public, it is unclear whether the company case studies truly represent the average capability of vaccine companies in middle-income developing countries to set up new production lines. What can be concluded from the case studies is that the four success cases demonstrate that *some* developing countries are able to develop, produce, and market a new influenza vaccine given eight years. However, the human, technical, and economic problems encountered by the other companies drive home the point that setting up new influenza vaccine production lines is time-consuming and is a high-risk endeavor from a business perspective.

15.9.3.2 US Vaccine Donations

The United States supports foreign seasonal and pandemic influenza vaccine stockpiles through direct vaccine donations, which represents a different pathway for the globalization of GoF benefits related to vaccine development and production. Specifically, any GoF-derived improvements to US vaccine development and production will indirectly benefit developed countries that receive US-produced vaccines through assistance and emergency response programs.

15.9.3.2.1 US Seasonal Vaccine Donations

The US Department of Health and Human Services' Centers for Disease Control has recently begun donating seasonal vaccines in an effort to increase seasonal influenza vaccination in developing countries.

The US CDC organizes the donation of seasonal influenza vaccines as part of the vaccine donation portion of the Partnership for Influenza Vaccine Introduction.[1849] A first donation cycle was conducted in 2012, whereby 375,000 doses of vaccine donated by Walgreens Company (US) led to the vaccination of 355,902 individuals in the Lao People's Democratic Republic.[1850] The program was expanded in 2013, with programs launched in Nicaragua and Uganda. Lao received 100,000 doses, and Nicaragua 35,000, in

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[1846] Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2014," 2014, p.1-82, http://www.dcvmn.org/IMG/pdf/dcvmn_directory_2014.pdf. Accessed July 7, 2015.

[1847] Ibid.

[1848] F. Marc LaForce, "Developing a Trivalent Live Attenuated Influenza Vaccine," presentation given at the Workshop on Business Modeling for Sustainable Influenza Vaccine Manufacturing, Washington, D.C., U.S.A., January 14-16, 2013, <http://www.who.int/influenza_vaccines_plan/resources/session_5_laforce.pdf>.

[1849] The Task Force for Global Health, "Partnership for Influenza Vaccine Introduction," <http://www.taskforce.org/our-work/projects/partnership-influenza-vaccine-introduction>.

[1850] Joseph Bresee, CDC, "Global Action Plan for Influenza Vaccines – II: CDC's Supportive Activities," GAP-II Partners Meeting, Dubai, United Arab Emirates, March 18, 2013, <http://www.who.int/phi/Day1_9_Bresee_GAP2_CDC_PM_Dubai2013.pdf>.

2013.[1851] Additional private donors that donated vaccines, supplies, or subsidized shipping services included bioCSL, Becton Dickinson and Company, and UPS.[1852] At the US national level, DOD provided assistance, in particular through the donation of 5,000 vaccine doses from US Air Force bases in Kadena, Japan.[1853]

Several factors significantly limit the impact of this program. First, the program relies on private donations from manufacturers which in turn are "based on [the] availability of excess vaccine supply" and are therefore unpredictable and potentially limited.[1854] Second, WHO guidelines stipulate that the vaccine must be licensed for use in the recipient country.[1855] The amount of time necessary for the initial license of a seasonal influenza vaccine in-country will vary by country but is generally a lengthy process (e.g., ten months in the US).[1856] As this timeframe is too long for a given seasonal vaccine donation to be licensed in time for flu season, vaccine donations must be matched with countries that already have the vaccines approved for use. However, since the countries that would benefit most from vaccine donations do not have domestic influenza production capabilities and weak public health systems, including regulatory infrastructure for MCMs, many lack approval for available influenza vaccines.[1857] And finally, there is a problem of timing, as the correct hemisphere vaccine (Northern or Southern) must be donated at the right time to match the recipient country's influenza season, which limits donation options.[1858] This timing issue is compounded by late commitment announcements.[1859] Since donators currently donate vaccine surplus, they can most easily provide stocks after the US influenza season, but this may be too late for potential recipient countries with similar influenza seasons. As a result, the range of countries that can receive US donations under these programs is greatly limited.

15.9.3.2.2 US Vaccine Donations in Response to a Pandemic

In the event of a pandemic, US national policy calls for donations of vaccines to the WHO for redistribution to developing countries. As a member state to the WHO Pandemic Influenza Preparedness Framework, the US is committed to supplying influenza vaccines to a WHO-maintained pandemic benefit-sharing system, which would then redistribute vaccines to developing countries as necessary to respond to a pandemic.[1860] The US HHS is the lead agency for the relinquishing of assets to international organizations in response to an outbreak. It, "in coordination with other United States Government Agencies, responds to requests for assistance from foreign countries and international organizations by contributing available HHS expertise and assets, including personnel and medical countermeasures (e.g., vaccines, antivirals and diagnostics)."[1861]

The exact quantity to be contributed by each member state is left for members to decide in the event of a

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1851 Alan R. Hinman, "Partnership for Influenza Vaccine Introduction (PIVI)," Dubai, United Arab Emirates, March 25, 2014, p.2, <http://www.who.int/phi/DAY1_08_Panel2_Hinman_Panel2_PIVI_PM_Dubai2014.pdf>.

1852 Centers for Disease Control and Prevention (CDC), "Laos and Nicaragua Protect High-Risk Persons from Influenza, with Help from Donor Coalition and CDC," <http://www.cdc.gov/flu/international/highlight-high-risk.htm>.

1853 The Task Force for Global Health, "Partnership for Influenza Vaccine Introduction (PIVI)," <http://www.taskforce.org/our-work/projects/partnership-influenza-vaccine-introduction>.

1854 Alan R. Hinman, "Partnership for Influenza Vaccine Introduction (PIVI)," p. 5.

1855 Ibid.

1856 World Health Organization (WHO), "Report of the Sixth Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers," p.21, <http://apps.who.int/iris/bitstream/10665/85515/1/9789241505994_eng.pdf>.

1857 Alan R. Hinman, "Partnership for Influenza Vaccine Introduction (PIVI)," p. 5.

1858 Ibid.

1859 Ibid.

1860 World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits* (Geneva: World Health Organization Press, 2011), p. 15-16, 18.

1861 U.S. Department of Health & Human Services, "North American Plan For Animal and Pandemic Influenza (NAPAPI)," April 2, 2012, p. 16.

pandemic; the WHO's guidance document calls for the provision of an "appropriate contribution to this system."[1862] The WHO guidance document, however, makes clear that the vaccine donations should be structured as a percentage of vaccine production runs, to ensure timely supply.[1863] The following case study, on the US vaccine donations in response to the 2009 H1N1 pandemic, show how and to what extent US vaccine donations can reach developing countries.

The 2009 pandemic preceded and motivated the formation of the WHO's Pandemic Influenza Preparedness Framework in 2011. As such, although the actions taken by the US during the pandemic remain instructive, certain shortcomings in the international donation and response system have been addressed by the establishment of a Framework.

15.9.3.2.3 Case Study: US Pandemic Vaccine Donations During the 2009 H1N1 Pandemic

During the H1N1 influenza pandemic, US vaccine donations were organized in response to 17 bilateral requests and a call for "global solidarity" from the WHO Director General.[1864] In September 2009, the United States pledged up to 10% of its vaccine production runs to the WHO; eight other countries subsequently made similar pledges.[1865,1866] The US H1N1 influenza response established a "10%" rule of thumb, whereby 10% of vaccine production runs would be donated to the WHO for distribution to developing countries in need of assistance.

The decision to relinquish vaccines to the WHO for international deployment was coordinated by the White House Security Staff International H1N1 Vaccine Assistance Working Group across several US agencies (i.e., this required more than HHS input).[1867] HHS has described the decision process as depending upon, *inter alia*:

- Vaccine supplies, in particular supplies available for international deployment,
- Domestic need and demand,
- Requests from WHO and bilateral requests,
- Legal authority to procure and deploy the vaccines,
- Available funding,
- The quantity and source of the required ancillary supplies, and
- Options for financing transportation and deployment.[1868]

The WHO served as the overall coordinator during the donation process, but USAID assisted in the development of country vaccination plans and with carrying out the necessary vaccination campaigns.[1869] In total, the United States donated 16,860,100 doses of 2009 H1N1 influenza vaccine to the WHO for international distribution, which represented approximately 14% of the vaccines committed to the

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1862 World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits*, p. 15-16, 18.
1863 Ibid.
1864 "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," p. 86, <http://www.phe.gov/Preparedness/mcm/h1n1-retrospective/Documents/h1n1-retrospective.pdf>.
1865 The eight countries were: Australia, Brazil, France, Italy, New Zealand, Norway, Switzerland, and the United Kingdom.
1866 World Health Organization, "Report of the WHO Pandemic Influenza A(H1N1) Vaccine Deployment Initiative," 2012, p. 4, <http://www.who.int/influenza_vaccines_plan/resources/h1n1_deployment_report.pdf>.
1867 Ibid.
1868 Ibid.
1869 Ibid.

WHO.[1870,1871] Out of a total of 122,450,000 vaccine doses committed by all states, the WHO distributed a total of 78,066,290 doses of vaccines to 77 countries.[1872]

Overall, donation of vaccines to the WHO suffered from severe timeliness issues. Vaccine production and domestic supply difficulties in the US (and other developed countries) in turn impacted vaccine donations. In October 2009, limited vaccine availability forced the US HHS Secretary to publicly announce that the US would delay the promised vaccine donations until the slated at-risk population in the US could be vaccinated.[1873,1874] (Notably, production of the vaccine was delayed due to difficulties in generating a high-yield vaccine strain that was suitable for large-scale production, a shortcoming that GoF research that enhances virus production aims to address.) Similar delays in promised deliveries could occur again, if future pandemic strains generate similarly low-yield vaccine strains. Advanced purchase agreements, whereby a given number of vaccines not yet produced are purchased by a government from a private vaccine producer, compounded accessibility issues.[1875] Since the vaccines already belonged to a particular buyer, the private firm was unable to donate a portion of the run to the WHO, regardless of a desire to do so.[1876]

Other developed countries were reticent in donating vaccines, and in a particularly severe pandemic whether promised doses would reach developing countries in time to be effective is unclear.[1877] For example, Canada's five million vaccine dose donation began only after the *second* wave of the flu pandemic was declared "over" in-country.[1878] Several developed countries– such as France, Germany, Switzerland, and the Netherlands– tried to sell excess vaccines instead of donating them.[1879,1880] For example, when only roughly five million people in France accepted the vaccine out of a stockpile order of 94 million doses, France attempted to sell its stocks at the same price it had obtained the vaccines.[1881] The WHO Pandemic Influenza Preparedness Framework's explicit clause on the provision of vaccines on a

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1870 United States of America, "Identifying and addressing barriers to the emergency sharing of international public health and medical assistance," Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, Meeting of Experts, Geneva, Switzerland, August 12-16, 2013, BWC/MSP/2013/MX/WP.6, p. 2 para. 5.

1871 "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," p. 87, <http://www.phe.gov/Preparedness/mcm/h1n1-retrospective/Documents/h1n1-retrospective.pdf>.

1872 The commitment of vaccines to the WHO involves a signed agreement, and therefore goes beyond a political pledge. World Health Organization, "Final Pandemic (H1N1) 2009 Vaccine Deployment Update," November 10, 2010, <http://www.who.int/csr/disease/swineflu/action/h1n1_vaccine_deployment_final_update_2010_11_10.pdf>.

1873 David P. Fidler, Kelly Lee, "Negotiating Equitable Access to Influenza Vaccines: Global Health Diplomacy and the Controversies Surrounding Avian Influenza H5N1 and Pandemic Influenza H1N1." *PLoS Med* 7, no. 5 (May 2010), <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2864298/>.

1874 Supriya Kumar et al., "US Public Support for Vaccine Donation to Poorer Countries in the 2009 H1N1 Pandemic," *PLoS One* 7, no. 3 (2012), <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3295778/>.

1875 Sam F. Halabi "Obstacles to pH1N1 Vaccine Availability: The Complex Contracting Relationship among Vaccine Manufacturers, the World Health Organization, Donor and Beneficiary Governments," *The Public Health Response to 2009 H1N1: A Systems Perspective*, eds. Michael A. Stoto, Melissa A. Hidgon (New York: Oxford University Press, 2015), p. 207.

1876 Ibid.

1877 David P. Fidler, Kelley Lee, "Negotiating Equitable Access to Influenza Vaccines: Global Health Diplomacy and the Controversies Surrounding Avian Influenza H5N1 and Pandemic Influenza H1N1."

1878 Supriya Kumar et al., "US Public Support for Vaccine Donation to Poorer Countries in the 2009 H1N1 Pandemic," *PLoS One* 7, no. 3 (2012), <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3295778/>.

1879 Ibid.

1880 "La France veut revendre ses vaccins contre la grippe A," [France wants to sell its vaccines against influenza A] *Le Parisien*, January 3, 2010, <http://www.leparisien.fr/societe/la-france-veut-revendre-ses-vaccins-contre-la-grippe-a-03-01-2010-763246.php>.

1881 Ibid.

rolling basis seeks to prevent this particular donation timeliness problem, but whether countries will comply with the Framework during a severe pandemic remains untested.[1882]

In addition to delays in the donation of vaccine doses, the planning and execution of the donation and distribution of vaccine doses and ancillary supplies was hampered by several factors that further delayed and/or reduced the quantity of vaccine doses distributed to recipient countries. The process by which US vaccines were donated and reached the end-users was negatively affected by "liability issues, vaccine registration requirements, and ensuring that recipient countries had in place funding and approved Vaccine Deployment and Vaccination Plans to support distribution of the vaccine."[1883] The WHO had to coordinate the vaccination plan in the recipient country with the US donor, and HHS diplomatically noted the existence of some US-WHO coordination friction by stating: "HHS and USAID […] remained in close contact in order to coordinate the deployment of vaccine, transport of vaccine and the deployment of ancillary items, though ultimate decisions on the recipient countries were made by the WHO based on their allocation procedures—thus, these decisions were not necessarily aligned."[1884] Since the nature and extent of these disagreements have not been revealed, it is difficult to ascertain their impact on the timeliness of donated vaccine availability.

In conclusion, roughly 14% of the WHO distributed vaccines during the H1N1 pandemic were donated by the United States, which collectively reached 77 recipient countries. The pandemic response suffered from serious timeliness issues with donations, coupled with logistical challenges during distribution and during in-country vaccination. These challenges highlight that, while US donation of vaccines is a viable pathway by which GoF benefits to vaccine production may globalize, the time needed to orchestrate the logistics of vaccine shipment and vaccination in-country will delay delivery of a vaccine to a developing country's population relative to a scenario in which that country is capable of indigenously producing and freely distributing its own vaccine doses.

15.9.3.3 Summary – Globalization Potential of GoF Benefits to Influenza Vaccine Production

GoF research has potential to benefit the production of vaccines in several ways: (1) through the development of higher-yield vaccine viruses, which shorten vaccine production timelines to enhance the availability and efficacy of vaccines, (2) through improving strain selection capabilities for seasonal influenza vaccines, which improves vaccine efficacy by increasing the likelihood of vaccine match, and (3) through the identification of molecular markers for enhanced virulence and antiviral resistance, which can be removed from vaccine strains to enhance the safety of the vaccine production process. These benefits can be realized by developing countries in two ways: (1) through the direct application of GoF research insights to production in-country and (2) through the receipt of US-produced vaccines donated through assistance or emergency response programs.

With respect to indigenous production capabilities, both the total number of vaccine *producers* outside of high-income countries (17) and the number of non-high income producing *countries* (7) has increased since 2010. As WHOCCs provide ready access to candidate vaccine strains to all such producers, these countries are currently capable of harnessing GoF research benefits to vaccine production. The total number of producers outside of high-income countries is slated to increase by as many as an additional six countries given current R&D efforts by at least 13 companies spanning a total of eight non high-income countries. Analysis of the R&D timelines for foreign influenza vaccine manufacturers that

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[1882] World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits*, p. 15-16, 18.

[1883] World Health Organization, "Final Pandemic (H1N1) 2009 Vaccine Deployment Update," November 10, 2010, <http://www.who.int/csr/disease/swineflu/action/h1n1_vaccine_deployment_final_update_2010_11_10.pdf>.

[1884] Ibid.

received BARDA funding support in 2006 shows that bringing a new influenza vaccine to market may require up to eight years.

This analysis revealed significant challenges associated with the establishment of new influenza vaccine production lines in developing countries, as only four of the 13 producers that received funding in 2006 are currently actively producing influenza vaccines. Impediments to the establishment of production lines include human factors (e.g., alleged corruption delaying construction of manufacturing facilities), technical factors (e.g., contamination of vaccine doses), and economic factors (e.g., lack of domestic demand). Lack of demand for influenza vaccines in-country appears to be a particularly important issue facing all producers, which is compounded by a lack of knowledge about optimal vaccination strategies, with respect to vaccine composition and the timing of vaccine delivery, in tropical regions. Therefore, whether current R&D efforts for the establishment of new production lines will come to fruition is uncertain, and the rate of continued development of new production capabilities in the future cannot be ascertained.

US donations of pandemic or seasonal flu vaccines provide a second pathway for GoF-derived benefits to reach developing countries. The US experience during the 2009 H1N1 pandemic demonstrated that, although the US was committed to providing some 10% of its vaccine stocks to developing countries through the WHO, the effectiveness of these donations suffered from serious timeliness issues. Although the WHO Pandemic Influenza Preparedness Framework (developed in 2011) established guidelines for vaccine donation during a pandemic in an effort to address these shortcomings, the ability of the US and the WHO to provide donated vaccines in time to mitigate the effects of a high morbidity influenza pandemic in the world's developing countries remains unverified. The US CDC organizes the donation of surplus seasonal influenza vaccines from vaccine manufacturers to developing countries, but several factors significantly limit the impact of this program, including the need for donated vaccines to be licensed in the recipient country and mismatches between the timing of vaccine availability and the needs of recipient countries.

15.9.4 Potential Benefit 2- Assistance in the Development of New Influenza or Coronavirus Antivirals

Several types of GoF research have the potential to inform the development of new influenza or coronavirus antivirals, namely GoF research that alters host tropism, that enhances pathogenicity, and that leads to evasion of antivirals. Here we briefly summarize how GoF research outputs benefit the development of new therapeutics. For a detailed analysis of each GoF benefit, refer to individual benefit sections devoted to the benefits of each GoF phenotype above.

First, GoF approaches that enhance the virulence of influenza viruses or coronaviruses may lead to the identification of novel virulence factors that are good therapeutic targets, thereby enabling the development of novel therapeutics.

Second, GoF approaches that alter the host range of CoVs enable the development of mouse-adapted virus strains. Unlike other animal models for CoVs, infection of mice with mouse-adapted CoV strains mimics the pathology of human disease; thus mouse-adapted strains serve as a robust system for testing the safety and efficacy of candidate therapeutics. Notably, because mouse-adapted strains are the only model system that satisfies the FDA Animal Efficacy Rule, the use of mouse-adapted strains is essential for the licensure of new CoV therapeutics in the US.

Third, GoF approaches that lead to evasion of therapeutics inform the development of new therapeutics for influenza viruses and coronaviruses. Specifically, these approaches provide insight into the

mechanism of action of therapeutics and demonstrate the genetic threshold for acquisition of resistance (i.e., the number of mutations that are required to gain resistance), which speaks to the potential field efficacy of the therapeutic. Both types of data are recommended for inclusion in an Investigational New Drug (IND) application to the FDA, thus this approach also plays a critical role in the licensure of new therapeutics in the US.

These benefits may be harnessed by developing countries either through indigenous production of new antivirals, or through direct US donations of antivirals in the event of a pandemic.

15.9.4.1 Capacity for Foreign Production of GoF-Derived New Influenza Antivirals

Several developing countries produce antivirals against influenza that were originally developed in high-income countries, including the US Several countries also conduct research on novel influenza antiviral candidates originally discovered in developed countries.

The process by which a pharmaceutical company abroad can proceed to produce an antiviral compound discovered in the US is complex. When a novel compound showing medical promise is developed into a potential treatment by scientists working for a company, the company typically owns the rights to the discovery as per the scientists' contracts and is then free to patent the potential treatment. For instance, scientists at Gilead Sciences discovered what would become the influenza antiviral medication Tamiflu, and Gilead Sciences held the patent on Tamiflu.[1885]

Since patents are filed at the national level, certain US pharmaceutical companies' compounds are not patent protected in certain countries that nevertheless have domestic antiviral pharmaceutical production capabilities. For instance, Tamiflu is not patent protected in Thailand, the Philippines, and Indonesia.[1886] Pharmaceutical companies based in these countries are therefore free to produce the underlying active compound of Tamiflu (oseltamivir) as a generic medication, provided that no additional bilateral or multilateral trade agreement clauses prohibits this activity.

For countries where a US patent is legally valid or where a US invention has been patented in-country, domestic producers can either obtain a license or challenge the patent's validity by producing the compound without a license. The licensing process allows a patent holder to include limits and conditions that it otherwise could not impose by simply selling the patent, such as the requirement to sell the product within specific geographic confines (such as a single country).[1887] Gilead Sciences, for example, licensed their compound's patent to the pharmaceutical company Roche as part of a co-development agreement for Tamiflu signed in 1996; the amended license agreement text reportedly allows Gilead Sciences to play a role in Roche's oversight of the compound's manufacture and commercialization and its pandemic planning for the product.[1888,1889] In practice, however, firms are often reluctant to license production in order to maintain production line exclusivity, and governmental and public pressure has played a role in convincing US firms to grant licenses to foreign companies. Roche was for instance threatened by several Congress representatives with a temporary abrogation of the Tamiflu patent when the firm was unable to meet demand during the 2005 H5N1 pandemic preparedness period, after which the company reached a

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[1885] Brian T. Yeh, "Influenza Antiviral Drugs and Patent Law Issues," CRS Report for Congress, August 16, 2007, p. 7, retrieved at <http://www.ipmall.info/hosted_resources/crs/RL33159_070816.pdf>.
[1886] Roche, "Factsheet Tamiflu," November 17, 2006, p.6. <http://www.roche.com/tamiflu_factsheet.pdf>.
[1887] Brian T. Yeh, "Influenza Antiviral Drugs and Patent Law Issues," p. 7.
[1888] Ibid.
[1889] Gilead Sciences Inc., "Gilead and Roche End Tamiflu® Dispute; Expanded Collaboration Includes Gilead Role in Oversight of Manufacturing and Commercialization," November 16, 2005, <http://investors.gilead.com/phoenix.zhtml?c=69964&p=irol-newsArticle&ID=783456>.

number of sub-licensing agreements with other companies abroad to produce the compound.[1890] Indeed, national patent law traditionally allows governments to cancel medication patents or to force the licensing of the compounds in response to medical emergencies.[1891] In cases where the applicability of a patent is disputed or unclear and where there is a potential emergency need, governments may simply decide not to attempt to enforce patent laws. The chairman of the Indian company Cipla alluded to such a situation when he declared in 2005 at the height of the shortage issues around the antiviral that, "Right or wrong, we're going to commercialize and make oseltamivir."[1892]

Patents protect a product for a significant period of time. The first US patent covering Tamiflu, for instance, was filed in 1996 by Gilead Sciences, and the company is still fighting in court attempts to produce generic oseltamivir medication by referencing its patent protections.[1893,1894] Once associated patents on a compound and its manufacturing expire, all competitors are allowed to produce the compound as a generic medication.[1895]

The following section focuses on the ability of foreign countries to establish production lines for notional novel influenza or coronavirus antivirals developed in the United States with assistance from GoF research. Deriving benefits from such a US discovery, however, goes beyond the foreign country's ability to establish a production line. It will also crucially depend on its ability to negotiate the complex patent issues noted above.

Current patent and licensing laws are in a state of flux, as a result of growing public and governmental pressure for affordable medication at the national level and as a result of comprehensive multinational trading negotiations that would potentially make it easier for pharmaceutical companies to obtain patents and increase the ability of companies to sue governments over intellectual property losses.[1896] The following section draws lessons from recent cases of globalization of antiviral production lines, but these conclusions reflect the current policy landscape and may become less relevant if patenting and licensing laws significantly change in the future.

15.9.4.1.1 Capacity for Novel Influenza Antiviral Production Abroad

This section considers the capacity of developing countries to establish production lines for new antivirals developed with assistance from GoF research. The experience with the globalization of production capabilities for the existing influenza antivirals zanamivir, oseltamivir, and peramivir (approved for use in the US), as well as for laninamivir octanoate (approved for use in Japan) are used as case studies to estimate the length of time needed to establish production of a new antiviral. Of note, all four antivirals are small molecule compounds, and all were discovered in high-income (developed) countries.

As of 2015, zanamivir, oseltamivir, and peramivir, but not laninamivir octanoate, were approved for use

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[1890] Brian T. Yeh, "Influenza Antiviral Drugs and Patent Law Issues," p. 3-4.
[1891] Donald G. McNeil Jr., "Indian Company to Make Generic Version of Flu Drug Tamiflu," *The New York Times*, October 14, 2005, <http://www.nytimes.com/2005/10/14/health/indian-company-to-make-generic-version-of-flu-drug-tamiflu.html>.
[1892] Ibid.
[1893] Kali Hays, "Gilead Sues Lupin Over Plans To Produce Generic Tamiflu," *Law 360*, September 17, 2015, <http://www.law360.com/articles/703920/gilead-sues-lupin-over-plans-to-produce-generic-tamiflu>.
[1894] U.S. Patent 5,763,483 A, "Carbocyclic Compounds," Filed December 27, 1996, Published June 9, 1998, <http://www.google.com/patents/US5763483>.
[1895] World Health Organization (WHO), "Generic Drugs," <http://www.who.int/trade/glossary/story034/en/>.
[1896] "Hard pills to swallow," *The Economist*, January 4, 2014, <http://www.economist.com/news/international/21592655-drug-firms-have-new-medicines-and-patients-are-desperate-them-arguments-over>.

against influenza in the United States.[1897,1898] With the exception of the newly-discovered peramivir and laninamivir octanoate compounds, these antivirals were all listed in the 2004 WHO Guidelines on the Use of Vaccines and Antivirals During Influenza Pandemics.[1899] Table 15.41 below summarizes information on these antivirals.

[1897] Centers for Disease Control and Prevention (CDC), "Influenza Antiviral Medications: Summary for Clinicians," p.1, retrieved at "Antiviral Drugs: Recommendations of the Advisory Committee on Immunization Practices (ACIP) Information for Health Care Professionals," March 4, 2015, <http://www.cdc.gov/flu/pdf/professionals/antivirals/antiviral-summary-clinician.pdf>.

[1898] Ribavirin is mentioned in the literature but its effectiveness has been questioned. See: World Health Organization, "WHO Guidelines on the Use of Vaccines and Antivirals during Influenza Pandemics," WHO/CDS/CSR/RMD/2004.8, 2004, Annex 5, p. 3, <http://www.who.int/csr/resources/publications/influenza/11_29_01_A.pdf>.

[1899] World Health Organization, "WHO Guidelines on the Use of Vaccines and Antivirals during Influenza Pandemics," Annex 5, p. 3.

Table 15.41. Information on Influenza Antivirals

Generic name	Proprietary manufacturer [1900]	Brand name	Category	Year compound published	Earliest FDA approval, any formulation
Zanamivir	GlaxoSmithKline	Relenza	Neuraminidase inhibitors	1993.[1901]	July 1999.[1902]
Oseltamivir	Roche	Tamiflu	Neuraminidase inhibitors	1997.[1903]	October 1999.[1904]
Peramivir	Biocryst	Rapivab	Neuraminidase inhibitors	2000.[1905]	Emergency use in 2009, approved for use in December 2014.[1906]
Laninamivir octanoate	Biota Pharmaceuticals and Daiichi Sankyo	Inavir	Neuraminidase inhibitors	2009.[1907]	Currenily not FDA-approved; approved for use in Japan against Influenza A and B since 2010 and 2013, respectively.[1908]

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[1900] [WHO] Technical Studies Under Resolution WHA63.1, Final Document, A/PIP/OEWG/3/2, p. 117;
"Biota Reports That Laninamivir Octanoate is Approved for the Prevention of Influenza in Japan," *Biota*, December 20, 2013, <http://investors.biotapharma.com/releasedetail.cfm?releaseid=815483>.

[1901] Mark Von Itzstein et al., "Rational Design of potent sialidase-based inhibitors of influenza virus replication," *Nature* 363 (June 1993): p. 418-423, <http://www.nature.com/nature/journal/v363/n6428/abs/363418a0.html>.

[1902] U.S. Food and Drug Administration, "FDA Approved Drug Products: Drug Details, RELENZA," <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails>.

[1903] Kim C. U. et al., "Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity," *J. Am. Chem Soc.* (January 1997): p. 681-690, <http://www.ncbi.nlm.nih.gov/pubmed/16526129>.

[1904] U.S. Food and Drug Administration, "FDA Approved Drug Products: Drug Details, TAMIFLU," <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails>.

[1905] Babu Y.S. et al., "BCX-1812 (RWJ-270201): discovery of a novel, highly potent, orally active, and selective influenza neuraminidase inhibitor through structure-based drug design," *Journal of Medical Chemistry* 43, no. 19 (2000): p. 3482-3486.

[1906] U.S. Food and Drug Administration, "FDA approves Rapivab to treat flu infection," *FDA News Release*, December 22, 2014, <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427755.htm>.

[1907] Makoto Yamashita et al., "CS-8958, a Prodrug of the New Neuraminidase Inhibitor R-125489, Shows Long-Acting Anti-Influenza Virus Activity," *Antimicrobial Agents and Chemotherapy* 53, no. 1 (2009): p. 186-192.

[1908] Biota Pharmaceuticals, Inc., "Biota Provides Update on BARDA Contract for Laninamivir Octanoate," May 8, 2014, <http://investors.biotapharma.com/releasedetail.cfm?releaseid=846423>.

All four compounds have been produced by some middle-income developing countries. Since companies mostly do not report on R&D efforts nor publicize the terms regarding technology transfers of sublicenses, finding out the average length of time necessary to establish production capability for a given degree of technology assistance is very difficult. Efforts to develop production capabilities in developing countries can nevertheless be broadly grouped into three strategies: licensed activities coupled with follow-on research, independent ventures, and exploratory research. Some examples of companies in middle-income countries are given below for each strategy to qualitatively illustrate the challenges and timescale associated with each approach, although limited details are available for some cases.

Licensed Activities Coupled with Follow-On Research

Vietnam received permission from Roche to encapsulate the oseltamivir compound on November 9, 2005.[1909,1910] Vietnamese scientists, such as members of the Ha Noi University of Pharmacy and the Institute of Chemistry of the Vietnamese Institute of Science and Technology, have since been engaged in laboratory production of oseltamivir, and have also experimented with recycling the substance from expired tablets.[1911,1912,1913]

In China, the Shanghai Pharmaceutical Group and HEC Pharm Co. are the two companies licensed to supply the Chinese state with oseltamivir.[1914,1915] Under a restriction imposed by Roche, the producers can "only use it for pandemic purposes within China"; in practice, the firms were not allowed to sell the compound commercially and had to furnish oseltamivir to the state at regulated prices.[1916] Shanghai Pharmaceutical Group announced they could produce 200,000 doses in *six months* when they obtained their licensing agreement in December 2005.[1917] The amount of R&D time invested by the firm prior to December 2005 to establish this oseltamivir production capacity was not revealed, but the announcement came some eight years after oseltamivir was identified as a potential MCM in the published literature (1997).[1918]

1909 "Calls for more money as the threat looms ever larger," *The Economist*, November 11, 2005, <http://www.economist.com/node/5134571>.

1910 Vietnam Ministry of Foreign Affairs, "Viet Nam signs agreement on Tamiflu production with F.Hofmann-Laroche," August 10, 2005, <http://www.vietnamembassy-tanzania.org/en/vnemb.vn/tinkhac/ns051111100413>.

1911 "Vietnam likely to produce Tamiflu from anise next year," *Xinhua* through *People*, March 21, 2006, <http://en.people.cn/200603/21/eng20060321_252323.html>.

1912 "Viet Nam to Produce Tamiflu from Star Aniseed," *Talk Vietnam*, March 24, 2006, <http://www.talkvietnam.com/2006/03/viet-nam-to-produce-tamiflu-from-star-aniseed/>.

1913 "Scientists hope to recycle 10m out-of-date Tamiflu tablets," *Viet Nam News*, August 10, 2015, <http://vietnamnews.vn/social-issues/health/203702/scientists-hope-to-recycle-10m-out-of-date-tamiflu-tablets.html>.

1914 Kirby Chien, Devidutta Tripathy, "China, India drug firms say primed for swine flu," *Reuters*, April 30, 2009, <http://uk.reuters.com/article/2009/04/30/us-flu-drugs-generic-idUKTRE53T0UL20090430>.

1915 "Roche licenses China firm to produce Tamiflu," *China Daily*, December 12, 2005, p.1-2, <http://www.chinadaily.com.cn/english/doc/2005-12/12/content_502758.htm>.

1916 Roche opens Tamiflu to outside firms," *Swiss Info*, December 12, 2005, <http://www.swissinfo.ch/eng/roche-opens-tamiflu-to-outside-firms/4900404>.

1917 Wang Xu, "Shanghai firm wins license for generic version of Tamiflu," *China Daily*, December 13, 2005, <http://www.chinadaily.com.cn/english/cndy/2005-12/13/content_502775.htm>.

1918 Kim C. U. et al., "Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity," *J. Am. Chem Soc.* (January 1997), p. 681-690, <http://www.ncbi.nlm.nih.gov/pubmed/16526129>.

Also in China, the firm Nanjing Simcere Dongyuan Pharmaceutical Co. Ltd., a subsidiary of Simcere Pharmaceutical Group, obtained a license to produce and sell zanamivir in September 2006.[1919,1920] According to a Simcere spokesman, GlaxoSmithKline licensed the production of the drug but only provided "limited technical support" in its synthesis.[1921,1922,1923] A pathway was developed in-country through joint research with the Shanghai Institute of Materia Medica and the Nanjing EffectPharm Drug Development Corporation.[1924] The firm obtained approval from the Chinese national regulator to manufacture and sell the compound in China in 2010, and the firm is currently selling the compound.[1925] The firm has conducted innovative research for production lines related to zanamivir, since the Shanghai Institute of Materia Medica published papers in 2012 and 2013 on the design and synthesis of zanamivir analogs.[1926,1927]

In India, Hetero Drugs obtained a sublicense for the production and sale of oseltamivir in December 2005.[1928] The company has since supplied millions of tablets of oseltamivir to the Indian government.[1929,1930]

Independent Ventures

As noted above, the Indian company Cipla publicly announced in October 2005 that it would independently produce oseltamivir without entering into a commercial agreement with Roche.[1931] In a subsequent interview, the company chair declared that the company had begun researching oseltamivir production techniques in 2004.[1932] In a parallel effort, Cipla announced it was producing zanamivir without entering into a commercial agreement with GlaxoSmithKline in 2006.[1933,1934] In India today, Cipla Ltd., Ranbaxy Laboratories, Strides Arcolab, and Natco Pharma all have production capacity for

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1919 GlaxoSmithKline, "Agreement to increase availability of Zanamivir supply in Asia and Lease Developed Countries," May 15, 2007, <http://www.gsk-china.com/asp/News/client/newconten/515200791555.htm>.
1920 PR Newswire, "Simcere Receives SFDA Approval to Manufacture and Sell Zanamivir in China," *Bloomberg*, February 11, 2010, <http://www.bloomberg.com/apps/news?pid=21070001&sid=aRO5.9_34evg>.
1921 Ibid.
1922 "Scientists develop ways producing anti-bird flu drug Zanamivir," *People's Daily*, February 6, 2009, <http://en.people.cn/90001/90781/90878/6587151.html>.
1923 EffactPharm, "Research Progress," July 10, 2015, <http://www.effectpharm.com/yifang_e.html>.
1924 Shanghai Institute of Materia Medica, Chinese Academy of Sciences, "The New Drug Certificate for Anti-H1N1 Flu Medicine Zanamivir granted to SIMM," March 17, 2010, <http://english.simm.cas.cn/rp/201003/t20100317_51500.html>.
1925 Simcere, "Zanamivir," <http://www.simcere.com/english/products/detail.asp?gongs_id=59&leibieid=APIs>.
1926 Feng E, et al., "Structure-based design and synthesis of C-1 and C-4-modified analogs of zanamivir as neuraminidase inhibitors," *Journal of Medicinal Chemistry* 56, no. 3 (2013): p. 671-684.
1927 Ye. D. et al., "Synthesis of C-4-modified zanamivir analogs as neuraminidase inhibitors and their anti-AIV activities," *European Journal of Medical Chemistry* 54 (2012): p. 7640-770.
1928 "Roche grants Tamiflu licence to Hetero Drugs," *The Times of India*, December 24, 2005, <http://timesofindia.indiatimes.com/business/india-business/Roche-grants-Tamiflu-licence-to-Hetero-Drugs/articleshow/1344422.cms>.
1929 Khomba Singh, "Hetero bags mega chunk of govt's anti-flu drug deal," *The Economic Times*, May 5, 2009, <http://articles.economictimes.indiatimes.com/2009-05-05/news/27636779_1_hetero-drugs-anti-flu-drug-oseltamivir>.
1930 Gireesh Chandra Prasad, "Govt to buy bird flu drugs from Roche, Hetero," *The Economic Times*, December 7, 2005, <http://articles.economictimes.indiatimes.com/2005-12-07/news/27487189_1_hetero-drugs-bird-flu-task-force>.
1931 "The Tamiflu Manufacturing Controversy: An Interview with Yusuf Hamied," *Multinational Monitor* vol. 27, no. 2, March/April 2006, <http://www.multinationalmonitor.org/mm2006/032006/interview-hamied.html>.
1932 Ibid.
1933 Ibid.
1934 "Cipla MD favours compulsory licensing sans monopoly," The Hindu Business Line, November 15, 2005, <http://www.thehindubusinessline.com/todays-paper/tp-corporate/cipla-md-favours-compulsory-licensing-sans-monopoly/article2195410.ece>.

oseltamivir without having entered into an agreement with Roche.[1935,1936,1937,1938,1939]

Thailand took advantage of the fact that Tamiflu had not been patent-protected in-country and has had independent production capacity for the generic oseltamivir since 2006.[1940,1941,1942] The Governmental Pharmaceutical Organization manufactured 200,000 tablets in early February 2006, following an announcement that it would do so in December 2005.[1943]

Independent Exploratory Research

A number of research groups in developing countries publish research on synthesis pathway optimization for newly discovered antiviral compounds. The ultimate objective of this type of research may be to prepare for in-country industrial production of the antiviral in question, although end-use intent cannot be definitely predicted based on publications in the scientific literature.

The chemical compound peramivir (first published in 2000 and approved for emergency use in the US in 2009 and for general use in 2014) has already been synthesized in a novel process by a Chinese research team, which achieved this result by March 2012 at the latest.[1944] Unlike earlier publications that described known pathways to obtain peramivir that were funded through grants for basic research projects on new drugs,[1945,1946] the Chinese research team developed a new pathway designed for *industrial* production. This new research effort was funded by the Guangdong Production and Research Joint Project, which may indicate an interest in future Chinese domestic production of the compound.[1947] In a peer-reviewed paper that appeared in 2013, the team reported an improved synthetic route for peramivir synthesis with a total 34% yield that obviated the need for a highly toxic chemical in the final step.[1948] At a minimum, the publication's results demonstrate that domestic production of the compound is well within China's

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1935 "Resistant strain of swine flu feared; virus killing thousands in India," *Japan Times*, February 26, 2015, <http://www.japantimes.co.jp/news/2015/02/26/asia-pacific/science-health-asia-pacific/resistant-strain-of-swine-flu-feared-virus-killing-thousands-in-india/#.VcjIdfnZViY>.

1936 "Swine flu: Hetero Healthcare increases Fluvir production by 400%," *The Economic Times*, February 26, 2015, <http://articles.economictimes.indiatimes.com/2015-02-26/news/59541921_1_swine-fluvir-oseltamivir>.

1937 Khomba Singh, "Govt curbs sale of flu drug Zanamivir," *The Economic Times*, August 29, 2009, <http://articles.economictimes.indiatimes.com/2009-08-29/news/28483297_1_swine-flu-drug-oseltamivir-zanamivir>.

1938 Kirby Chien, Devidutta Tripathy, "China, India drug firms say primed for swine flu," *Reuters*, April 30, 2009, <http://uk.reuters.com/article/2009/04/30/us-flu-drugs-generic-idUKTRE53T0UI20090430>.

1939 "Ranbaxy to supply oseltamivir capsules to US," *The Economic Times*, October 21, 2007, <http://articles.economictimes.indiatimes.com/2007-10-21/news/28461984_1_capsules-domestic-sales-generic-version>.

1940 "Tamiflu- Oseltamivir Production," *News Medical*, February 1, 2011, <http://www.news-medical.net/health/Tamiflu-Oseltamivir-Production.aspx>.

1941 Pennapa Hongthong, "Scientists produce generic Tamiflu," *The Nation*, August 4, 2006, <http://www.nationmultimedia.com/2006/08/04/national/national_30010320.php>.

1942 Roche, "Factsheet Tamiflu," November 17, 2006, p.6, <http://www.roche.com/tamiflu_factsheet.pdf>.

1943 CRS, International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses, http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1944 Fei Jia, Juan Hong, Ping-Hua Sun, Jian-Xin Chen, Wei-Min Chen, "Facile Synthesis of the Neuraminidase Inhibitor Peramivir," *Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry* 43, no. 19 (2013): p. 2641-2647, <http://www.tandfonline.com/doi/abs/10.1080/00397911.2012.729279>.

1945 顾轶娜,林东海,"新型抗流感病毒神经氨酸酶抑制剂帕拉米韦研究进展,"中国生化药物杂志 30, no. 4 (2009): p.273-276 [GU Yi-na, LIN Dong-Hai, "Research progress on peramivir as a novel anti-influenza virus neuraminidase inhibitor," *Chinese Journal of Biochemical Pharmaceutics* 30 no. 4 (2009): p.273-276.]

1946 贾飞, 陈良柱, 陈建新, 孙平华, 陈卫民,"帕拉米韦合成路线图解,"中国医药工业杂志 42 no. 12 (2011): p. 954-956. [JIA Fei, CHEN Jianxin, SUN Pinghua, CHEN Weimin, "Graphical Synthetic Routes of Peramivir," *Chinese Journal of Pharmaceuticals* 42, no. 12 (2011): p. 954-956.]

1947 Fei Jia et al., "Facile Synthesis of the Neuraminidase Inhibitor Peramivir," p. 2646.

1948 Ibid, p. 2641.

technical capabilities. The peramivir case is one in which a novel synthetic pathway for a US designed chemical was rapidly developed abroad, indeed even before the compound was approved for general use in the US by the FDA.

Similarly, in December 2014, a Chinese research team working out of the State Key Laboratory of Bioorganic and Natural Products Chemistry of the Shanghai Institute of Organic Chemistry published a novel synthetic pathway for the production of laninamivir octanoate.[1949] This published process used an inexpensive acid as a starting compound and reportedly obtained a 72% total yield with a 12-step process that was suitable for scale-up.[1950] Overall, the paper's process effectively lowers industrial production costs while minimizing losses in yield and minimizing the number of additional industrial steps required, which are three extremely important factors necessary for industrial scale-up.

As demonstrated by these accounts, indigenous zanamivir and oseltamivir production lines exist in several middle-income countries. Several Chinese research groups have also demonstrated the capability to efficiently synthesize peramivir and laninamivir octanoate, raising the possibility that in-country production lines could be rapidly set up should the decision to do so be made. Although the amount of R&D time invested by each of the companies and research teams named above to achieve their production capability is unknown (i.e., when the company began researching synthetic pathways and/or began setting up production facilities), conservative estimates demonstrate that at least some middle-income countries achieved the capacity for full-scale production of a given MCM less than ten years after the compound was initially published in the literature. In the case of laninamivir octanoate, a Chinese laboratory demonstrated a novel chemical synthesis process for the compound less than five years after the compound was published in the literature. Notably, several companies rapidly activated production capabilities capable of producing hundreds of thousands of doses in less than six months in 2005– 2006 when their governments were preparing for a potential H5N1 pandemic. This suggests that, as with influenza vaccines, a general lack of demand for influenza antivirals appears to be keeping production line globalization in check. Based on these cases, the actual time needed to initiate commercial production of an antiviral designed in a developed country appears to be in the one to five year range.

In conclusion, should GoF research enable the development of a new promising small molecule antiviral compound targeting influenza viruses or coronaviruses, the experience with current antiviral compounds suggests that at least some developing countries have the will and the means to develop methods for production of a potential MCM in-country. In turn, this production capability could then be scaled-up to industrial production once the compounds can be legally produced either through license or as generics, improving global pandemic response capabilities. We note that although barriers to the establishment of production lines may vary between different types of therapeutics (e.g., small molecule drugs versus monoclonal antibodies), patenting and licensing issues are likely to be the same for all types of therapeutics.

15.9.4.2 US Antiviral Donations

GoF benefits to the development of novel antivirals may also globalize through US donations of antivirals to developing countries. Current US government assistance to antiviral supply abroad are primarily limited to plans for donations to the WHO for redistribution to developing countries in case of an influenza pandemic. As a member state in the WHO Pandemic Influenza Preparedness Framework, the United States government is committed to contributing influenza antivirals to the WHO-organized Pandemic Influenza Preparedness Benefit Sharing System, which would redistribute MCMs to third

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[1949] Tian J. et al., "Organocatalytic and scalable synthesis of the anti-influenza drugs zanamivir, laninamivir, and CS-8958," *Angewandte Chemie* 126 (2014): p. 14105-14108.
[1950] Ibid, p. 14105-14106.

countries as part of a pandemic response as needed.[1951] US private pharmaceutical companies can and have donated antiviral treatments to the WHO and to countries dealing with local outbreaks independently from government contributions.[1952,1953] However, these private companies are under no obligation to do so in the future, and hence the effect of this potential GOF-derived benefits dissemination pathway cannot be reliably assessed.

As there are no licensed therapeutics for coronaviruses in the US or abroad, neither the US nor the WHO have formal policies or plans in place for the donation of (notional) therapeutics in the event of an epidemic caused by a novel coronavirus.

The following case study reviews US donations of antivirals to foreign countries during the 2009 H1N1 pandemic and identifies bottlenecks that may pose a barrier to the globalization of GoF benefits via this pathway in the future. Although the creation of the WHO Pandemic Influenza Preparedness (PIP) Framework in 2011 limits the extent to which this case study is predictive of the successes and challenges of influenza antiviral donation efforts in the future given its plan for a joint pre-pandemic influenza antivirals stockpile,[1954] similar challenges could be encountered in the event of ad hoc donation of CoV therapeutics during a CoV epidemic.

15.9.4.3 Case Study: US Antiviral Donations During the 2009 H1N1 Pandemic

The comprehensive after-action report, "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," does not expound on the US decision to disburse antivirals to other nations beyond noting that it was carried out by HHS "after careful consideration of federal policies and discussions of global demand."[1955] Responding to the 2009 H1N1 pandemic was initially an ad-hoc process, given the lack of a uniform US decision-making process at the start of the pandemic. The need for such a process proved to be a major lessons-learned from the pandemic. Given that a US national framework for decision-making has since been developed, this shortcoming is less likely to hamper international donation and distribution of influenza antivirals in the event of a future pandemic. This national framework applies to all "international requests for public health emergency medical countermeasures,"[1956] and hence would also apply during a hypothetical CoV pandemic.

The US initially gave 400,000 antiviral treatment courses to Mexico, followed by 420,000 courses of oseltamivir for the Pan American Health Organization.[1957] The Pan American Health Organization then provided stocks to countries throughout Latin America and the Caribbean.[1958] Although this demonstrates US willingness to provide antiviral doses in the event of a pandemic, one US public health policy stakeholder stated that the global health security enterprise may not be as willing to donate antivirals in

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[1951] World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits*, p. 15-16, 18.

[1952] David Reddy, "Responding to pandemic (H1N1) 2009 influenza: the role of oseltamivir," *J. Antimicrob. Chemother.* 65 supplement 2 (April 2010): ii35-ii40, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2835510/pdf/dkq014.pdf>.

[1953] Roche, "Factsheet Tamiflu," November 17, 2006, p.6, <http://www.roche.com/tamiflu_factsheet.pdf>.

[1954] World Health Organization (WHO), Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits (Geneva: World Health Organization Press, 2011), p. 18, <http://apps.who.int/iris/bitstream/10665/44796/1/9789241503082_eng.pdf>.

[1955] "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," p. 38.

[1956] Public Health Emergency, U.S. Department of Health & Human Services, "International Assistance and Response Policy Branch," October 16, 2014, <http://www.phe.gov/about/OPP/dihs/Pages/policy.aspx>.

[1957] "An HHS Retrospective on the 2009 H1N1 Influenza Pandemic to Advance All Hazards Preparedness," p. 38, <http://www.phe.gov/Preparedness/mcm/h1n1-retrospective/Documents/h1n1-retrospective.pdf>.

[1958] United States of America, "Identifying and addressing barriers to the emergency sharing of international public health and medical assistance," Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, Meeting of Experts, Geneva, Switzerland, August 12-16, 2013, BWC/MSP/2013/MX/WP.6, p. 2 para. 5.

the event of future pandemics due to the expense associated with storing and deploying the drugs.[1959]

The use of donated antivirals during the H1N1 pandemic in developing countries was in general suboptimal, in part due to the low availability of the antiviral compounds.[1960] In Asia for instance, an authoritative review article noted that, "health practitioners were reluctant to follow the recommendation of the empiric use of oseltamivir": the practitioners did not wish to use scarce doses on ostensibly mild cases of influenza, even when the patient was in a high-risk group.[1961]

In sum, although US policy supports the donation of influenza antivirals in the event of a pandemic, the relatively small number of doses donated in comparison to the global need in the event of a pandemic means that developing countries would face shortages, which would in turn exacerbate poor usage in-country.

15.9.4.4 Summary – Globalization Potential of GoF Benefits to Influenza Vaccine Production

GoF research has the potential to benefit the development of novel therapeutics for influenza viruses and coronaviruses in several ways. First, GoF research that enhances virulence enables the identification of novel virulence factors, which may be good therapeutic targets. Second, mouse models for CoVs, developed through GoF approaches that alter host range, represent a robust system for testing the safety and efficacy of therapeutics in development. Third, GoF approaches that lead to evasion of therapeutics also support the licensure of new therapeutics by providing information that is critical for an Investigational New Drug application to the FDA.

The ability of developing countries to establish production lines for novel influenza or hypothetical coronavirus therapeutics depend not only on their manufacturing capabilities but also on their ability to negotiate the complex patent issues surrounding the marketing of therapeutics. In cases where patent protections do not apply, analysis of the timeline and circumstances surrounding the establishment of production lines for existing influenza antivirals in several developing countries suggest that the time needed to initiate commercial production of a US-designed or commercialized antiviral is one to five years. Patent protections do not apply when a patent is not recognized nationally or is abrogated during a medical emergency, or where the compound can be sublicensed from the patent owner. Notably, several companies in developing countries rapidly activated influenza antiviral production capabilities to produce hundreds of thousands of doses in less than six months in 2005—2006, when their governments were preparing for a potential H5N1 pandemic. This capacity for rapid scale-up of production suggests that the actual time needed for establishment of a new production line may be much less than five years. As with influenza vaccines, a general lack of domestic demand for influenza antivirals appears to be keeping globalization of GoF benefits related to the development of novel therapeutics in check.

The US demonstrated its willingness to donate antivirals during the 2009 H1N1 pandemic. However, problems of timeliness of supply compounded issues of suboptimal use in-country. The WHO Pandemic Influenza Preparedness Framework (developed in 2011) addresses these shortcomings but remains untested.

[1959] (2015g) Interview with US government official involved in public health preparedness and response decision-making for influenza outbreaks.

[1960] Dale Fisher et al. "Pandemic response lessons from influenza H1N1 2009 in Asia." *Respirology* 16 (2011): p. 879. <http://onlinelibrary.wiley.com/doi/10.1111/j.1440-1843.2011.02003.x/abstract>.

[1961] Ibid.

15.9.5 Potential Benefit 3- Benefits to Pandemic Preparedness Planning

This section assesses the globalization of GoF benefits that inform pandemic preparedness planning, which includes two benefits. First, the demonstration that avian influenza viruses can evolve the capacity for more efficient transmission in mammals may, in and of itself, stimulate interest and investment in pandemic preparedness initiatives. Second, molecular markers for phenotypic properties of concern (e.g., virulence, transmissibility, mammalian adaptation, and antiviral resistance), which are discovered and validated using GoF approaches, inform pandemic risk assessments that guide prioritization of resources for pandemic preparedness activities. The first benefit derives from GoF research that enhances the transmissibility of influenza viruses in mammals; the second derives from GoF research that enhances the infectivity or transmissibility of influenza viruses in mammals, that enhances the virulence of influenza viruses, and that leads to evasion of influenza viruses from therapeutics. For a detailed analysis of these GoF benefits, refer to individual benefit sections for each GoF phenotype.

Formal and informal pandemic risk assessments inform the extent to which governments invest in pandemic preparedness and response activities as well as how those resources are directed, given that many zoonotic influenza strains pose potential risks to human populations. These activities include enhanced surveillance and implementation of interventions at the animal-human interface to mitigate risks of disease spillover into human populations, in order to bolster prevention and early detection capabilities, as well as development of pre-pandemic vaccines.

The extent to which GoF benefits to pandemic risk assessments will globalize depends on several factors:

- Whether and how information gleaned from GoF studies influences risk assessments and decision-making about pandemic preparedness activities in developing countries in which high-risk animal influenza viruses are currently circulating,
- Whether those countries have the ability to successfully implement community-level interventions that mitigate the risk of disease spillover into human populations and that bolster their capacity for early detection of potential spillover events, or
- Whether those countries have the capacity to produce pre-pandemic vaccines in-country.

In this section, we evaluate current capabilities and challenges for each factor in turn.

15.9.5.1 Role of GoF Research in Pandemic Risk Assessments for Developing Countries

This section evaluates whether and how information gleaned from GoF studies influences risk assessments and pandemic preparedness planning in developing countries in which high-risk animal influenza viruses are currently circulating. Two types of GoF studies are considered: (1) ""proof of principle" demonstrations that particular animal influenza viruses can acquire pandemic properties (e.g., transmissibility) in the laboratory and (2) studies that establish molecular markers for phenotypic properties of concern (transmissibility, virulence, etc.).

Although "proof of principle" experiments that demonstrate that an avian virus (e.g., H5N1) can acquire the capacity for more efficient transmission in mammals have had minimal impacts on USG initiatives due to the already high investments in pandemic preparedness, these GoF results have relatively greater impacts on preparedness efforts in developing countries. One international public health official stated

that the experimental demonstration that H5N1 could evolve the capacity for airborne transmission in ferrets was of "great importance" in countries where H5N1 was circulating.[1962,1963,1964] In response, some countries mounted communications campaigns to engage with the public, public health personnel, and health care workers about the risks associated with H5N1, in an effort to bolster their surveillance capabilities. Thus to date, these GoF experiments primarily benefit global rather than domestic populations.

As discussed in detail in Section 15.3.5.2, risk assessments of particular virus strains integrate several different types of information that influence the pandemic potential of a virus, including information about the transmissibility, virulence, and other properties of the virus, information about pre-existing immunity and other properties of the host population, and information about the circulation of the virus in local animal populations and other ecological factors. Most developing countries in which animal influenza viruses of concern (e.g., H5N1) are circulating are not capable of conducting ferret experiments to evaluate the transmissibility and virulence of viruses, which contribute critical data to a pandemic risk assessment.[1965] As a result, those developing countries carry out risk assessments in conjunction with the WHO (as well as the CDC and other laboratories in the GISRS as needed).[1966] This collaborative relationship is codified in the WHO's Pandemic Influenza Preparedness Benefit Sharing System, which states that WHO will seek to ensure that member states and the WHO Secretariat "provide pandemic surveillance and risk assessment and early warning information and services to all countries."[1967] These assessments are conducted with input from the Ministries of Health in a country of interest.[1968] In addition to conducting risk assessments when new viruses of concern emerge, the WHO regularly updates previous risk assessments in light of new epidemiologic observations and new data that has been generated since the previous assessment. Similar to risk assessments conducted by the USG, WHO risk assessments consider the presence of molecular markers of mammalian adaptation, transmissibility, and virulence, alongside virological data and in the context of environmental factors that play important roles in the emergence of pandemic viruses.

Ultimately, the ability of a developing country to derive benefits from risk assessments informed by GoF research will depend on the ability of the country to engage in responsive pandemic preparedness activities. These include enhanced surveillance, implementation of community-level risk mitigation measures, and pre-pandemic vaccine development.[1969] The following sections assess the potential for developing countries to put in place such "downstream" responses.

15.9.5.2 Capacity for Responsive Public Health Preparedness Measures in Foreign Countries

Responsive capabilities are primarily relevant in countries in which zoonotic influenza viruses (or influenza viruses with zoonotic potential) are currently circulating. As seen on the map below (Figure 15.4), most countries in the world have detected cases of zoonotic avian influenza in humans or in birds

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1962 Herfst S *et al* (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 336: 1534-1541

1963 Imai M *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486: 420-428

1964 (2015f) Interview with international researcher or international public health official.

1965 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

1966 Ibid.

1967 World Health Organization (WHO), *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits*, p.15.

1968 (2015a) Interview with U.S. CDC (Centers for Disease Control) representative.

1969 C. Todd Davis et al., "Use of Highly Pathogenic Avian Influenza A(H5N1) Gain-Of-Function Studies for Molecular-Based Surveillance and Pandemic Preparedness," *mBio* 5, no. 6 (December 12, 2014) <http://mbio.asm.org/content/5/6/e02431-14.full>.

within the last five years. Notably, a lack of detected cases may be due to poor detection and reporting capabilities rather than the absence of avian influenza.[1970]

Figure 15.4. Countries that reported a detected case of zoonotic influenza in humans or birds within the last five years.[1971,1972,1973,1974]

Many countries with AI detections are developing (low- or middle-income) countries, in particular most countries with repeated detections (i.e., multiple years) and sustained outbreaks in domestic poultry populations. Public health responses to zoonotic influenza outbreaks in developing countries are particularly challenging due to limited resources for carrying out response activities and because of the need for a strong and coordinated veterinary service– public health system. The veterinary services of most developing countries greatly suffer from weak human organizational factors compounded by resource constraints.[1975] The lack of effective communication strategies for behavioral interventions that will reduce risks of disease spillover (e.g., at poultry farms, live bird markets, etc.) was also highlighted by influenza researchers and public health experts as a major challenge.[1976] Convincing the public to comply with disruptive measures is difficult, and one expert noted the value of GoF research results in

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1970 Tiaji Salaam-Blyther, "The 2009 Influenza Pandemic: U.S. Responses to Global Human Cases," Congressional Research Service, June 23, 2009, p. 11, <https://www.acs.org/content/dam/acsorg/policy/acsonthehill/globalchallengesdiscussions/swineflu/crs-r40588-us-responses.pdf>.

1971 H5N1, H5N6, H6N1, H7N2, H7N3, H7N7, H7N9, H9N2, H10N7, H10N8.

1972 World Health Organization (WHO), "Disease Outbreak News (DONs)," <http://www.who.int/csr/don/en/>.

1973 World Health Organization (WHO), "Monthly Risk Assessment Summary, Influenza at the Human-Animal Interface," <http://www.who.int/influenza/human_animal_interface/HAI_Risk_Assessment/en/>.

1974 Food and Agriculture Organization of the United States, "EMPRES-i Global Animal Disease Information System," <http://empres-i.fao.org/eipws3g/>.

1975 J. Weaver et al., "Initial assessment of strategic plans for improving the performance of Veterinary Services in developing countries: a review of OIE PVS Gap Analysis reports," *Rev. sci. tech. Off. int. Epiz.* 32, no. 2 (2012): p. 631-645.

1976 (2015f) Interview with international researcher or international public health official.

strengthening the evidence basis for recommendations. Nevertheless, as the following cases will highlight, developing countries can still mount a public health response in the face of zoonotic influenza detections.

The following section presents comparative case studies of the public health response in Thailand, Vietnam, and Laos to novel influenza infections in people. Thailand is an upper-middle-income economy, while Vietnam and Laos are lower-middle-income economies.[1977] All three countries had cases of highly pathogenic H5N1 infections in humans and in domestic poultry starting in late 2003 and early 2004.[1978] The purpose of these case studies is to highlight the successes and challenges associated with implementing community-level interventions to respond to the presence of "risky" influenza viruses circulating in the native animal population.

The case studies demonstrate the overarching importance of a strong public health sector in being able to benefit from pandemic risk assessments through implementation of prevention activities. The cases of Thailand and to some degree Vietnam showcase that a robust response to a significant public health risk in middle-income countries is not impossible. The case of Laos is instructive in demonstrating that, for countries with little initial public health surveillance, the benefits realized from implementing community-level response measures downstream of a pandemic risk assessment can be marginal at best.

15.9.5.2.1 Case Studies: Thailand, Vietnam, and Laos and the 2004 H5N1 Outbreaks

The following comparative case studies showcase different public health responses to the 2004 H5N1 outbreaks in Vietnam, Thailand, and Laos. Vietnam was the first of the three countries to report cases in humans, shortly followed by Thailand and eventually by Laos. Vietnam's response was resource-intensive but initially ad hoc, with mixed success. Thailand's approach was all-encompassing, with good success. Laos had virtually no response capabilities in 2004 and its response mostly focused on establishing a national surveillance system.

Vietnam

Vietnam first reported H5N1 in poultry on January 8, 2004 and in humans on January 11, 2004.[1979] Vietnam initially responded to the 2004 cases with ad hoc bird eradication and poultry movement restrictions.[1980] These measures proved ineffective, given the lack of nationwide surveillance and coordinated response capabilities. In response, Vietnam launched a nation-wide surveillance effort in 2004, and that year, 2272 samples were collected from poultry, of which 515 tested positive for HPAI H5N1.[1981,1982] Detection sampling was greatly expanded in 2005, with 13,889 samples collected from poultry of which 1,317 tested positive.[1983]

1977 The World Bank, "Country and Lending Groups," http://data.worldbank.org/about/country-and-lending-groups. Accessed July 7, 2015.

1978 David A. Boltz et al., "H5N1 Influenza Viruses in Lao People's Democratic Republic," *Emerging Infectious Diseases* (2006): p. 1593, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3290961/>.

1979 World Health Organization (WHO), "H5N1 avian influenza: Timeline of major events," January 25, 2012, p.1, <http://www.who.int/influenza/human_animal_interface/H5N1_avian_influenza_update.pdf>.

1980 Ricardo J. Soares Magalhaes, Dirk U. Pfeiffer, Joachim Otte, "Evaluating the control of HPAIV H5N1 in Vietnam: virus transmission within infected flocks reported before and after vaccination," *BMC Vet Res.* 6 (2010): p.1 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2898779/pdf/1746-6148-6-31.pdf>.

1981 Xiu-Feng Wan et al., "Evolution of Highly Pathogenic H5N1 Avian Influenza Viruses in Vietnam between 2001 and 2007," *PloSOne* 3, no. 10 (October 2008): 1-12, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2565130/pdf/pone.0003462.pdf>.

1982 See Table 1 in: Ibid.

1983 Ibid.

In addition to initiating enhanced surveillance, Vietnam's response strategy heavily relied on vaccination.[1984] A mass vaccination program for poultry was launched in August 2005, and the government announced in January 2006 that over 240 million birds had been vaccinated.[1985] The measures were not entirely successful. Specific challenges highlighted by Vietnamese practitioners included a "lack of knowledge about viral behaviors, pathogenicity, transmission mechanism, [and] treatment," problems with recognition and reporting systems, insufficient collaboration between human and animal health sectors, and a general lack of resources to implement "active surveillance and research."[1986] Today, H5N1 is considered endemic in poultry in Vietnam, and sporadic cases of human infection with H5N1 continue to be reported by Vietnam.[1987]

Thailand

Thailand was hard-hit by the emergence of H5N1 in-country, and suffered several fatalities.[1988] Mass die-offs at poultry farms in central and northern Thailand were noted starting in late 2003.[1989] Through mid-December 2003 to early 2004, neighboring countries such as China, Vietnam, Japan, and South Korea reported H5N1 outbreaks.[1990] In response to these reports, the Thai government deployed a human-case surveillance program in December 2003, followed by a poultry surveillance program in mid-January 2004.[1991] The human-focused effort identified 12 confirmed and 21 suspected influenza cases in country through polymerase chain reaction and viral isolation of respiratory specimens taken from individuals exhibiting symptoms similar to influenza.[1992] The animal-focused effort focused on collecting cloacal swabs from poultry farms throughout the country and led to the official announcement of the discovery of H5 HPAI in a chicken farm.[1993] The Thai national reference laboratory announced the first cases in both human and poultry on January 23, 2004.[1994] Subsequent monitoring results retrospectively analyzing poultry outbreaks in 144 villages made it clear that H5N1 had already been present in Thailand since the end of 2003.[1995]

Although initially lambasted by the local press for its sluggish response, the Thai government put in place

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1984 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1985 Ibid.

1986 Nguyen Tran Hien, "Avian Influenza In Vietnam: Situation and Lessons Learned," p.17, <http://www.fao.org/docs/eims/upload/250718/aj167e00.pdf>.

1987 Sharmi W. Thor et al., "Detection and Characterization of Clade 1 Reassortant H5N1 Viruses Isolated from Human Cases in Vietnam during 2013," *PloS One* 10, no. 8 (2015): p. 1-20, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4526568/pdf/pone.0133867.pdf>.

1988 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1989 Thanawat Tiensin et al., "Highly Pathogenic Avian Influenza H5N1, Thailand, 2004," *Emerging Infectious Diseases* 11, no. 11 (November 2005): p.1664-1672, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3367332/>.

1990 World Health Organization (WHO), "H5N1 avian influenza: Timeline of major events."

1991 Thanawat Tiensin et al., "Highly Pathogenic Avian Influenza H5N1, Thailand, 2004," *Emerging Infectious Diseases* 11, no. 11 (November 2005), <http://wwwnc.cdc.gov/eid/article/11/11/05-0608_article>.

1992 Tawee Chotpitayasunondh et al., "Human Disease from Influenza A (H5N1), Thailand, 2004," Emerging Infectious Diseases 11, no. 2 (February 2005): p. 201-209, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3320461/>

1993 Thanawat Tiensin et al., "Highly Pathogenic Avian Influenza H5N1, Thailand, 2004."

1994 Ibid.

1995 Ibid.

aggressive measures in an attempt to eradicate the virus.[1996] A systematic nation-wide survey to detect infections was rolled out in October 2004.[1997] Poultry and related products (e.g., feed, bedding, waste, and manure) were destroyed by veterinary authorities upon identification of the virus; over 40 million birds were reported killed in the nation-wide campaign.[1998,1999] Controls were placed on the movement of commercial poultry and fighting cocks and were enforced through mobile checkpoints set up in the most affected provinces.[2000] Finally, oseltamivir tablets were produced and sold at subsidized prices, starting with 200,000 tablets manufactured in February 2006.[2001]

As a result of these response measures, the last reported human case of avian influenza in Thailand was in 2006 and the last reported animal case of avian influenza was in 2008.[2002,2003,2004]

Laos

Laos first reported H5N1 in poultry one day following Vietnam's announcement, in January 27, 2004. The first reported human case was detected two years later, with an onset date of February 10, 2007.[2005] Prior to the 2004 H5N1 cases in humans in neighboring Vietnam and Thailand, Laos had extremely limited disease surveillance system. Select hospitals were operating an "early warning outbreak recognition" system using phones and faxes, but the information was not shared with the country's Epidemiology Unit.[2006] As a result, the unit was unable to implement pre-emptive measures to the 2004 outbreak.[2007] The response appears to have been limited to the culling of some 98,000 birds at commercial farms.[2008]

The country sought financial assistance abroad to implement country-wide public health reforms.[2009,2010] Laos deployed a disease surveillance network starting in 2007, with three surveillance stations for influenza-like-illnesses and one surveillance station for both influenza-like-illnesses and severe acute respiratory illnesses.[2011] The system was expanded in 2009, 2010, and 2011.[2012] Electronic means replaced

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1996 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

1997 David A. Boltz et al., "H5N1 Influenza Viruses in Lao People's Democratic Republic," p. 1593.

1998 Thanawat Tiensin et al., "Highly Pathogenic Avian Influenza H5N1, Thailand, 2004";

1999 CRS. CRS Report for Congress. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed January 26, 2016.

2000 Ibid, p. 17-18.

2001 Ibid, p. 17.

2002 Ibid.

2003 OIE, World Animal Health Organization Database (WAHID), "Detailed Country(ies) disease incidence," <http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail>.

2004 Food and Agriculture Organization of the United States, "EMPRES-i Global Animal Disease Information System," <http://empres-i.fao.org/eipws3g/>.

2005 World Health Organization (WHO), "H5N1 avian influenza: Timeline of major events," January 25, 2012, p.16, <http://www.who.int/influenza/human_animal_interface/H5N1_avian_influenza_update.pdf>.

2006 Bounlay Phommasack et al., "Capacity Building in Response to Pandemic Influenza Threats: Lao PDR Case Study," *Am. J. Trop. Med. Hyg.* 87, no. 6 (December 2012): p. 965-971, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3516098/>.

2007 Ibid.

2008 CRS. International Efforts to Control the Spread of the Avian Influenza (H5N1) Virus: Affected Countries' Responses. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL33349.pdf. Last Update August 24, 2006. Accessed March 15, 2016.

2009 Ibid, p. 16.

2010 The World Bank, "Facility supports a coordinated and effective response to H5N1 in Lao PDR (English)," <http://documents.worldbank.org/curated/en/2010/03/13160390/facility-supports-coordinated-effective-response-h5n1-lao-pdr>.

2011 Bounlay Phommasack et al., "Capacity Building in Response to Pandemic Influenza Threats: Lao PDR Case Study."

2012 Ibid.

the phone-and-fax communication system for the hospitals, while a phone-in system was rolled out so that rural areas could report cases to the national health authorities.[2013] Since the 2004 H5N1 cases and up until 2011, 19 H5N1 outbreaks in poultry have been detected in Laos.[2014] Since the rollout of the human-monitoring system in 2007 and up until 2011, a total of 31 influenza-like illness outbreaks in humans have been investigated; of these, 27 were confirmed as influenza cases.[2015] Laos has either been relatively spared from H5N1 cases in humans, with the last human case reported to WHO in 2007, or has had low case detection.[2016] H5N1 has not been reported in-country since mid-2010, and the recent emergence of H5N6 in poultry is due to a strain believed to have originated from China rather than emerging from Laos.[2017]

These cases are instructive in determining whether a developing country could benefit from utilizing pandemic risk assessments to prioritize response capabilities. Countries like Thailand, and to a lesser extent Vietnam, have demonstrated the ability to mount public health responses in the event of a serious health situation. Conversely, the downstream benefits of pandemic risk assessments are significantly limited in developing countries that lack the means to implement prevention and enhanced surveillance activities, such as the situation in Laos in 2004.

15.9.5.3 Capacity for Pre-Pandemic Vaccine Production

In addition to implementing community-level prevention and surveillance activities in response to a high-risk pandemic risk assessment, developing countries could derive benefits from such assessments by investing in pre-pandemic vaccine development and stockpiling. The influenza vaccine producers with influenza vaccines on the market identified in developing countries (see Section 16.9.6) are all capable of producing pandemic vaccine strains using CVVs obtained through the WHO framework, as explained in Section 16.9.3.1 above. The map in Figure 15.5 shows an overlay of the developing countries with current vaccine production capabilities and those in which zoonotic influenza viruses have been detected in bird and/or human populations within the past five years. Only seven out of 28 developing countries with zoonotic AI detections in humans or in bird populations over the past five years have the capacity to produce vaccines in-country. This result highlights that a limited number of countries that may be at risk of the emergence of a novel pandemic strain within their borders can benefit from pandemic risk assessments through the development and stockpiling of pre-pandemic vaccines. Notably, the WHO does not stockpile pre-pandemic vaccines for use in developing countries, but is rather focused on ensuring real-time access to pandemic vaccines during a pandemic as outlined in the Pandemic Influenza Preparedness Framework.[2018,2019]

2013 Ibid.
2014 Ibid.
2015 See Table 1 in: Ibid.
2016 The World Bank, "Disease Outbreak News- Lao People's Democratic Republic." <http://www.who.int/csr/don/archive/country/lao/en/>.
2017 Frank Y. K. Wong et al., "Reassortant Highly Pathogenic Influenza A(H5N6) Virus in Laos," *Emerging Infectious Diseases* 21, no. 3 (March 2015): p. 511-516.
2018 Immunizations SWGoIVa. Influenza A (H5N1) Vaccine Stockpile and Inter-Pandemic Vaccine Use Background Document. http://www.who.int/immunization/sage/meetings/2013/november/SAGE_WG_H5vaccine_background_paper_16Oct2013_v4.pdf. Last Update Accessed October 31, 2015.
2019 World Health Organization, *Pandemic influenza preparedness Framework for the sharing of influenza viruses and access to vaccines and other benefits* (Geneva: World Health Organization Press, 2011), p. 15-16, 18.

Figure 15.5. Overlay of low- and middle-income countries with current or planned influenza vaccine production capabilities and those that have reported AI detections in birds to OIE within the past five years. Regions with AI detections are outlined in red. Countries (or regions) without vaccine production capabilities are shaded in white, countries with current vaccine production capabilities are shaded in dark blue, and countries with planned vaccine production lines are shaded in cyan.

15.9.5.4 Summary – Globalization of GoF Benefits That Inform Pandemic Risk Assessments

The demonstration that animal influenza viruses can acquire pandemic properties in a laboratory setting may galvanize preparedness efforts in developing countries where the virus is circulating in agricultural animal or wildlife populations. For example, the 2012 demonstration that H5N1 could evolve the capacity for airborne transmission between ferrets triggered some developing countries to initiate communications campaigns to raise awareness of the risks associated with H5N1 infections among the public, public health personnel, and healthcare workers, in order to bolster early detection capabilities.

Because most developing countries in which high-risk animal influenza viruses are circulating lack the capabilities to conduct ferret experiments evaluating the transmissibility and virulence of viruses, data which critically inform pandemic risk assessments, risk assessments are carried out in collaboration with the WHO and laboratory members of the GISRS (including the CDC). Similar to USG risk assessments, these risk assessments incorporate information derived from GoF research, alongside epidemiologic and virologic data, and environmental factors that influence the pandemic potential of the virus.

Downstream of a pandemic risk assessment, the ability of developing countries to implement prevention and early detection measures in response to the detection of zoonotic influenza cases or outbreaks in humans and/or animals varies widely, depending on the state of public health infrastructure, the relationship between the Veterinary Services and Public Health sectors, and the resources for investing the prevention and response activities. Thailand's ability to eradicate H5N1 from their poultry production system in response to widespread outbreaks in poultry populations as well as multiple human spillover cases in 2003 – 2006 indicates that successful eradication campaigns are possible. However, the fact that Vietnam continues to experience HPAI outbreaks since the initial 2004 – 2005 outbreak in the region highlights the challenges for successfully carrying out response activities that mitigate the risk of avian influenza spillover into human populations.

Although multiple developing countries in which zoonotic avian influenza infections have been detected in human and/or bird populations within the past five years currently have the capacity to produce pre-pandemic influenza vaccines in-country, 21 do not. As the WHO does not stockpile pre-pandemic vaccines, the lack of vaccine production capabilities in some at-risk countries limits the globalization potential of GoF benefits related to pandemic risk assessments.

15.9.6 Information on Influenza Vaccine Production in Low- and Middle-Income Countries

The following dataset lists vaccine producers outside of high-income countries with influenza vaccine products on the market, with influenza vaccine R&D, or that formerly marketed influenza vaccines but appear to be no longer actively producing vaccines. The following list was compiled through several data sources:

- The 2011 WHO survey on global influenza production capacity, which identified 28 companies;[2020]
- The Developing Countries Vaccine Manufacturers Network (DCVMN) directories from 2014 and 2015, which list current and planned influenza vaccine manufacturer members;[2021,2022,2023]
- The International Federation of Pharmaceutical Manufacturers & Associations' Influenza Vaccine Supply Members list,[2024] and
- The US Department of Health and Human Services' Influenza Vaccine International Capacity Building Portfolio.[2025]

These were then supplemented by searches for potential manufacturers identified in the literature or in news reports.[2026]

2020 Jeffrey Partridge, Marie Paule Kieny, "Global production capacity of seasonal influenza vaccine in 2011," Vaccine 31, no. 5 (January 2013): p. 728-731, <http://www.sciencedirect.com/science/article/pii/S0264410X12015861>.

2021 While the DCVMN is a coordinating platform for vaccine producers in the developing world, certain DCVMN producers are in countries that are currently classed by the World Bank as being High-Income countries (such as South Korea).

2022 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p.1-96, <http://www.dcvmn.org/IMG/pdf/directory.pdf>.

2023 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2014," 2014, p.1-82, http://www.dcvmn.org/IMG/pdf/dcvmn_directory_2014.pdf. Accessed July 7, 2015;

2024 International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), "IFPMA Influenza task force – IVS Membership," <http://www.ifpma.org/resources/influenza-vaccines/ifpma-influenza-task-force/ivs-membership.html>

2025 U.S. Department of Health & Human Services, "International Influenza Vaccine Capacity Building Portfolio," <https://www.medicalcountermeasures.gov/projectmaps/Who.aspx>.

2026 Jan Hendriks, Yan Liang, Bing Zeng, "China's emerging vaccine industry," *Human Vaccines* 6, no. 7 (2010): p. 602-607, <http://www.tandfonline.com/doi/pdf/10.4161/hv.6.7.11933>.

In total, the products of 36 vaccine companies based outside of high-income countries were researched. Of these, 18 were found to be actively producing influenza vaccines, 13 had R&D work for such a product at various stages of completion, and five were apparently not currently producing or researching influenza vaccines. The following table summarizes these findings. It is unlikely to be a complete listing, given that few companies provide up-to-date information on vaccine R&D efforts at the pre-clinical trial stage. In addition, some uncertainties remain in cases where a product was recently on the market (2014) but does not currently appear on the company's products page, and no news or business articles were available to explain the absence. Indeed, the WHO relies on company survey data to gauge current and near-future influenza vaccine production.[2027]

[2027] World Health Organization, "Pandemic Influenza Preparedness (PIP) Framework 2013 Partnership Contribution Questionnaire Final Results (30 May 2014)," May 30, 2014, <http://www.who.int/influenza/pip/2013_PC_Final_Results_30May2014.pdf>

Table 15.42. Current Influenza Vaccine Production Outside of High-Income Countries (Known Current Producers Are Emphasized in Blue Text)					
Vaccine Producers	**Current influenza vaccine producer?**	**Country**	**World Bank Income Ranking**	**Vaccine Network Association**	**Source**
Acerca de Birmex	No / R&D ?	Mexico	Upper-middle	DCVMN, BARDA/WHO	2028,2029
Amson Vaccines & Pharma (pvt) Ltd	Yes	Pakistan	Lower-middle		2030
Beijing Minhai Biotechnology	R&D	China	Upper-middle	DCVMN	2031,2032
Beijing Tiantan Biological Products	Yes	China	Upper-middle	DCVMN	2033
Bharat Biotech International Limited	Yes	India	Lower-middle	DCVMN	2034
Bio Farma	Yes	Indonesia	Lower-middle	DCVMN, BARDA	2035
Cadila Pharmaceuticals Limited	Yes	India	Lower-middle	DCVMN	2036
Cantacuzino Institute	No	Romania	Upper-middle	BARDA/WHO	2037
Changchun BCHT Biotechnology	R&D	China	Upper-middle	DCVMN, BARDA/WHO	2038,2039
Changchun Changsheng Life Sciences Limited	Yes	China	Upper-middle		2040

2028 No influenza product is explicitly listed in the company's entry in the DCVMN 2015 directory, unlike what was stated in the DCVMN 2014 directory. See: Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 47-48.

2029 Erika Hernández, "Producirá México vacuna contra influenza," [Mexico will produce an influenza vaccine], *Reforma*, July 13, 2015, <http://www.reforma.com/aplicacioneslibre/preacceso/articulo/default.aspx?id=590386&urlredirect=http://www.reforma.com/aplicaciones/articulo/default.aspx?id=590386>.

2030 Amson Vaccines & Amson Pharma (PVT) LTD., "Product Profile," <http://www.amson.org.pk/products.html>.

2031 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 3-4.

2032 Minhai Biotechnology Co. Ltd., "Patents," <http://en.biominhai.com/yfdt/&FrontComContent_list01-1369617220497ContId=56b25fd5-73e3-411e-8894-ab9462fc265e&comContentId=56b25fd5-73e3-411e-8894-ab9462fc265e.html>.

2033 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 5-6.

2034 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 7-8.

2035 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 11-12.

2036 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 19-20.

2037 "Institutul Cantacuzino nu face vaccin antigripal nici in sezonul 2015 - 2016, desi are autorizatii" [Cantacuzino Institute will not make flu vaccine in the 2015-2016 season, despite having licenses], *Ziare*, May 21, 2015, http://www.ziare.com/social/spital/institutul-cantacuzino-nu-face-vaccin-antigripal-nici-in-sezonul-2015-2016-desi-are-autorizatii-1364363. Accessed October 1, 2015.

2038 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 23-24.

2039 PATH, "Signing of new Letter of Agreement between BCHT and PATH supports influenza vaccine development in China," <http://sites.path.org/vaccinedevelopment/files/2015/02/BCHTbulletin-on-agreement-with-PATH_020215_for-web-no-watermark.pdf>.

2040 Changsheng, "Influenza Split Vaccine," <http://www.cs-vaccine.com/en/cp_page.asp?id=328>.

Table 15.42. Current Influenza Vaccine Production Outside of High-Income Countries (Known Current Producers Are Emphasized in Blue Text)

Vaccine Producers	Current influenza vaccine producer?	Country	World Bank Income Ranking	Vaccine Network Association	Source
China National Biotec Group	Yes	China	Upper-middle	DCVMN	2041
Dalian Aleph Biomedical	No	China	Upper-middle		2042
Dalian Hissen Bio-pharm	Yes	China	Upper-middle		2043
GPO	R&D	Thailand	Upper-middle	BARDA/WHO	2044
Hualan Biological Engineering	Yes	China	Upper-middle	IVS	2045
Incepta Vaccine Ltd	Yes	Bangladesh	Lower-middle	DCVMN	2046
Instituto Butantan	Yes	Brazil	Upper-middle	DCVMN, BARDA/WHO	2047
Institute of Vaccines and Medical Biologicals (IVAC)	R&D	Vietnam	Lower-middle	DCVMN, BARDA	2048,2049
Institute of Medical Biology, Chinese Academy of Medical Sciences	R&D	China	Upper-middle	DCVMN	2050
Jiangsu Ealong Biotech	No	China	Upper-middle		2051
Panacea Biotec Limited	Yes	India	Lower-middle	DCVMN	2052

2041 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 25-26.
2042 The company was acquired by Shanghai Fosun Pharmaceutical Co., Ltd. See: "Dalian Aleph Biomedical Co., Ltd.," *CMO CRO*, <http://www.cmocro.com/company/Dalian+Aleph+Biomedical+Co.,+Ltd./index.html>.
2043 Hissen, "产品中心: 流感病毒裂解疫苗 (2014/2015) 使用说明" ["Products: Influenza Virus Vaccine (2014/2015) Description"], <http://www.hissen.com/products/View.aspx?id=185>.
2044 "Vaccine factory to restart construction," *Bangkok Post*, December 11, 2014, <http://www.bangkokpost.com/lite/news/448902/vaccine-factory-to-restart-construction>.
2045 "Hualan is first influenza vaccine manufacturer in China to get WHO approval," *Vaccine News Daily*, June 17, 2015, <http://vaccinenewsdaily.com/stories/510549688-hualan-is-first-influenza-vaccine-manufacturer-in-china-to-get-who-approval>.
2046 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 37-38.
2047 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 43-44.
2048 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 41-42.
2049 Thanhniemews, "Affordable bird flu vaccine made in Vietnam passes first human trial," *Talk Vietnam*, April 23, 2015, <http://www.talkvietnam.com/2015/04/affordable-bird-flu-vaccine-made-in-vietnam-passes-first-human-trial/>.
2050 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 45-46.
2051 "It also halted production of the A/H1N1 flu vaccine in February when the quality permit expired, he said." In: "Two Chinese Drug Makers Halt Production," *CRI English*, April 1, 2010, <http://english.cri.cn/6909/2010/04/01/1461s560685.htm>.
2052 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 59-60.

Table 15.42. Current Influenza Vaccine Production Outside of High-Income Countries (Known Current Producers Are Emphasized in Blue Text)					
Vaccine Producers	**Current influenza vaccine producer?**	**Country**	**World Bank Income Ranking**	**Vaccine Network Association**	**Source**
Production & Research Complex for Pasteur Institute of Iran	R&D	Iran	Upper-middle	DCVMN	2053
Queen Saovabha Memorial Institute, The Thai Red Cross Society	R&D	Thailand	Upper-middle	DCVMN	2054
Razi Vaccine & Serum Research Institute	R&D	Iran	Upper-middle	DCVMN	2055
Research Institute for Biological Safety Problems (RIBSP)	Yes	Kazakhstan	Upper-middle	BARDA/WHO	2056
Serum Institute of India Ltd.	Yes	India	Lower-middle	DCVMN, BARDA/WHO	2057
Shanghai Fosun Pharmaceutical	Yes	China	Upper-middle		2058
Shenzhen Neptunus Interlong Biotech Co., Ltd.	Yes	China	Upper-middle		2059, 2060
Sinovac Biotech Ltd.	Yes	China	Upper-middle	DCVMN	2061
The Biovac Institute	Yes	South Africa	Upper-middle	DCVMN, BARDA/WHO	2062
The Government Pharmaceutical Organization	R&D	Thailand	Upper-middle	DCVMN	2063

2053 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 61-62.
2054 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 63-64.
2055 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 67-68.
2056 "Реестр отечественных поставщиков товаров фармацевтической и медицинской промышленности" [Register of domestic suppliers of goods Pharmaceutical and Medical Industries], March 13, 2015, <http://arkalyk.kostanay.gov.kz/uploads/files/1d03853ecd302518be6a42def9ca184a.doc>.
2057 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 67-68.
2058 FosunPharma, "产业布局 > 核心产品 > 疫苗," ["Industrial Distribution – Core Products – Vaccine"] <http://www.fosunpharma.com/products/ym>.
2059 Neptunus, "Company Profile," <http://www.interlong.com/En/About/>.
2060 China Commodity Net, "Shenzhen Neptunus Interlong Bio-technique Co., Ltd. – Submit Influenza Vaccine," <http://ccne.mofcom.gov.cn/493005>.
2061 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 71-72.
2062 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2014," 2014, p.1-82, http://www.dcvmn.org/IMG/pdf/dcvmn_directory_2014.pdf. Accessed July 7, 2015.
2063 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 81-82.

Table 15.42. Current Influenza Vaccine Production Outside of High-Income Countries (Known Current Producers Are Emphasized in Blue Text)					
Vaccine Producers	**Current influenza vaccine producer?**	**Country**	**World Bank Income Ranking**	**Vaccine Network Association**	**Source**
Tianjin Tasly Pharma	No	China	Upper-middle		2064,2065
Torlak Institute (Institute of Virology, Vaccines and Sera)	R&D	Serbia	Upper-middle	BARDA/WHO	2066
The Company for Vaccine and Biological Production No.1 (VABIOTECH)	R&D	Vietnam	Lower-middle	DCVMN, BARDA	2067,2068, 2069
VACSERA	R&D	Egypt	Lower-middle	DCVMN, BARDA/WHO	2070
Walvax Biotechnology	R&D	China	Upper-middle	DCVMN	2071,2072

2064 "Tasly setting up flu vaccine base in Tianjin," *Research In China*, July 31, 2007, <http://www.researchinchina.com/news/NewsInfo.aspx?id=6428>.
2065 Tasly Holding Group Co. Ltd., "Products." <http://www.tasly.com/en_web/Product_list2.aspx>.
2066 World Health Organization (WHO), Public Health Innovation and Intellectual Property (PHI), Department of Essential Medicines and Health Products (EMP), "Clinical Research Organization (CRO) to support an Inactivated Influenza Vaccine Clinical Trial in Serbia," Request for Proposal Bid Reference 2015/HIS/PHI/001, p. 1-33, <http://www.who.int/phi/news/RFP_2015_HIS_PHI_001.pdf>.
2067 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 79-80.
2068 Juliet Bryant, "Influenza vaccine manufacturing in Viet Nam: Report on the APACI Satellite session," *One Health*, 2015, <http://onehealth.org.vn/influenza-vaccine-manufacturing-in-viet-namreport-on-the-apaci-satellite-session new>.
2069 VABIOTECH, "Products – Vaccine," <http://www.en.vabiotech.com.vn/index.php?option=com_content&view=article&id=88&Itemid=109&lang=en>.
2070 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 83-84.
2071 Developing Countries Vaccine Manufacturers Network, "DCVMN Directory 2015," 2015, p. 87-88.
2072 Walvax Biotechnology Co. Ltd., "产品宣传册," ["Product Brochure"] <http://www.walvax.com/Model/6.aspx>.

15.10 List of Subject Matter Experts Interviewed for the Benefit Assessment

Table 15.43. List of Research Laboratories Visited

Principal Investigator	Department	Research Institution	Research Focus
Mark Denison	Departments of Pediatrics and Pathology, Microbiology, and Immunology	Vanderbilt University	Coronaviruses
Ralph Baric	Department of Epidemiology	University of North Carolina	Coronaviruses
Richard Webby	Infectious Diseases Department	St. Jude Children's Research Hospital	Influenza viruses
Charles Russell	Infectious Diseases Department	St. Jude Children's Research Hospital	Influenza viruses
Stacey Schultz-Cherry	Infectious Diseases Department	St. Jude Children's Research Hospital	Influenza viruses
Paul Thomas	Immunology Department	St. Jude Children's Research Hospital	
Kanta Subbarao	National Institute of Allergy and Infectious Diseases	National Institutes of Health	Coronaviruses and influenza viruses
Yoshihiro Kawaoka	Department of Pathobiological Sciences	University of Wisconsin, Madison	Influenza viruses
Adolfo Garcia-Sastre	Department of Microbiology	Mt. Sinai Hospital	Influenza viruses
Melissa Uccelini	Department of Microbiology	Mt. Sinai Hospital	Influenza viruses
Randy Albrecht	Department of Microbiology	Mt. Sinai Hospital	Influenza viruses
Walter Orenstein	Department of Medicine, Division of Infectious Diseases	Emory University	Influenza viruses
Anice Lowen	Department of Microbiology and Immunology	Emory University	Influenza viruses
John Steel	Department of Microbiology and Immunology	Emory University	Influenza viruses

In addition to the principle investigator listed above, postdoctoral fellows and other senior research staff, graduate students, and/or laboratory technicians were interviewed during each site visit.

Table 15.44. List of Additional Stakeholders Interviewed

Name	Title	Institute	Sector
Bright, Rick	Acting Director of the Influenza Division	HHS/ASPR/BARDA	Government
Cox, Nancy	Former Director of CDC's Influenza Division, Former Director of CDC's WHO Collaborating Center for Influenza	CDC	Government
Donabedian, Armen	Scientific Technical Advisor and Chief, Late Stage Development	HHS/ASPR/BARDA/Influenza Division	Government
Donis, Ruben	Associate Director for Policy, Evaluation, and Preparedness	Influenza Division, CDC	Government
Katz, Jackie	Acting Deputy Director	Influenza Division, CDC	Government
Korch, George	Senior Science Advisor	HHS/ASPR	Government
Meltzer, Martin	Lead, Health Economics and Modeling Unit	CDC	Government
Morens, David	Senior Advisor to the Director of NIAID	National Institutes of Health	Government
Robinson, Robin	Director of BARDA	HHS/ASPR/BARDA	Government
Rose, Patrick	Director, Pandemic and Catastrophic Preparedness	National Association of County and City Health Officials	Government
Roth, Cathy	Advisor, Office of the Assistant Director-General, Health Systems and Innovation Cluster	World Health Organization	Government
Vannieuwenhoven, Ty	Chief Veterinary Officer, National Disaster Medical System	HHS/ASPR/OEM	Government
Dormitzer, Phil	Vice President and Chief Scientific Officer: Viral Vaccines	Pfizer Vaccines Research and Development	Industry
Mahmoud, Adel	Senior Policy Analyst, Woodrow Wilson School of Public and International Affairs; Lecturer in Molecular Biology; Board of Directors, Inovio Pharmaceuticals	Princeton University; Inovio Pharmaceuticals	Industry

Table 15.44. List of Additional Stakeholders Interviewed			
Name	**Title**	**Institute**	**Sector**
Plotkin, Stanley	Executive Advisor	Sanofi Pasteur	Industry
Smith, Gale	Vice President of Vaccine Development	Novavax	Industry
Frieman, Matthew	Associate Professor, Department of Microbiology and Immunology	University of Maryland School of Medicine	Coronavirus researcher
Perlman, Stanley	Professor of Microbiology	University of Iowa	Coronavirus researcher
Poon, Leo	Associate Professor, Faculty of Medicine; School of Public Health	University of Hong Kong	Coronavirus and influenza researcher
Bennink, Jack	Section Chief	Laboratory of Viral Diseases/NIAID/NIH	Influenza researcher
Bowman, Andrew	Assistant Professor of Veterinary Preventive Medicine	Animal Influenza Ecology and Epidemiology Research Program, The Ohio State University	Influenza researcher
Brooke, Chris	Postdoctoral Fellow	Laboratory of Viral Diseases/NIAID/NIH	Influenza researcher
Bucher, Doris	Associate Professor of Microbiology and Immunology	New York Medical College	Influenza researcher
Fouchier, Ron	Professor of Virology	Erasmus University Medical Center	Influenza researcher
Hall, Jeffrey	Research Virologist	National Wildlife Health Center, USGS	Influenza researcher
Heise, Mark	Professor of Genetics	University of North Carolina School of Medicine	Influenza researcher
Ip, Hon	Microbiologist	Virology Laboratory, National Wildlife Health Center, USGS	Influenza researcher
Palese, Peter	Professor and Chair of Microbiology; Professor of Medicine, Infectious Diseases	Mount Sinai Hospital	Influenza researcher
Russell, Colin	Royal Society University Research Fellow, Principal Research Associate; Department of Veterinary Medicine	University of Cambridge	Influenza researcher

Table 15.44. List of Additional Stakeholders Interviewed			
Name	**Title**	**Institute**	**Sector**
Schwemmle, Martin	Professor of Virology	Institute of Virology and Department of Medical Microbiology and Hygiene, Albert Ludwig University of Freiburg	Influenza researcher
Smith, Derek	Professor of Infectious Disease Informatics; Director of WHO Collaborating Center for Modeling, Evolution, and Control of Emerging Infectious Diseases	University of Cambridge	Influenza researcher
Swayne, David	Center Director	US National Poultry Research Center, Agricultural Research Service, USDA	Influenza researcher
Taubenberger, Jeffery	Section Chief	Laboratory of Infectious Diseases/NIAID/NIH	Influenza researcher
Yewdell, Jonathan	Section Chief	Laboratory of Viral Diseases/NIAID/NIH	Influenza researcher
Casadevall, Arturo	Bloomberg Distinguished Professor	Johns Hopkins University	Non-PPP research
Duprex, Paul	Professor of Microbiology; Director of Cell and Tissue Imaging	Boston University School of Medicine; National Emerging Infectious Diseases Institute	Non-PPP research
Fraser, Christophe	Professor of Theoretical Epidemiology	Imperial College London	Non-PPP research
Imperiale, Michael	Professor and Associate Chair, Department of Microbiology and Immunology	University of Michigan Medical School	Non-PPP research
Kobinger, Gary	Head of Special Pathogens, Head of Vector Design and Immunotherapy; Special Pathogens Program; National Microbiology Laboratory	Public Health Agency of Canada	Non-PPP research
Lipsitch, Marc	Professor of Epidemiology	Harvard T.H. Chan School of Public Health	Non-PPP research

Table 15.44. List of Additional Stakeholders Interviewed

Name	Title	Institute	Sector
Relman, David	Professor of Microbiology and Immunology; Co-Director of the Center for International Security and Cooperation	Stanford University	Non-PPP research
Inglesby, Tom	Chief Executive Officer and Director	Center for Health Security, University of Pittsburgh Medical Center	Non-PPP research, clinician

16 Appendix V: Findings Informing the Biosecurity Risk Assessment

16.1 Purpose of the Biosecurity Risk Assessment (RA)

The purpose of the semi-quantitative biosecurity RA is to provide information regarding the risk that malicious actors would misuse the fruits of GoF research or intentionally cause an outbreak of engineered strains. The risk of malicious actors acquiring pathogens for use as a weapon and the risk of accidental infection caused by a malicious act (i.e., the release of infected animals from a laboratory) were considered in the assessment. Given that biosecurity risk has two distinct components each with unique vulnerabilities and consequences (malicious acts directed at a laboratory conducting GoF research and the misuse of information generated by GoF research), our approach to these components is discussed separately. For the biosecurity RA of malicious acts directed at a laboratory, the results are described as semi-quantitative biosecurity risk information that can be understood in the context of, and relative to, the quantitative biosafety risk information provided in the task described above. That is, this report will highlight which threats pose as much risk as accidents and which types of biosecurity measures (security systems or controls on information) are as critical to consider as the most important biosafety features.

No unclassified information describing the threats to research laboratories that store or study GoF influenza, SARS-CoV, or MERS-CoV virus is available. Therefore, to identify the types of malicious actors and acts that may target a GoF laboratory, the analysis included an examination of historical incidents involving life science laboratories and hospitals, evaluating the motivations and capabilities of malicious actors, and determining if and how existing security measures affect the likelihood of success of a malicious act.

Data collection included historical incidents within the past 25 years because information about incidents from prior years is not necessarily available or high quality, and the governance and oversight of research and life science laboratories differs from prior years. However, events beyond the 25 year mark were included only if sufficient, quality information was available and they provided relevant information about malicious actor interest, motivation, and/or capability, or malicious acts. This historical analysis provides an evidence-based method to understand, in a qualitative way, the probability that an event would occur and the type of resources these malicious actors bring to bear when targeting a laboratory.

Furthermore, all relevant information about laboratory biosafety and biosecurity security, whether codified or not, was collected to ensure the development of a complete picture of the governance and implementation of security and crossover safety measures at laboratories wherein GoF viruses are stored or studied. Seasonal influenza and MERS-CoV are not select agents. However, MERS-CoV is studied in biosafety level 3 (i.e., high containment) laboratories. Although the Biosafety in Microbiological and Biomedical Laboratories (BMBL) recommends currently circulating seasonal influenza be studied at biosafety level (BSL) 2 and not-currently circulating seasonal influenza be studied at higher containment, the actual level of containment is determined by the institution's biosafety risk assessment and requirements from appropriate regulatory agencies. In general, research with seasonal influenza is conducted at BSL-2, BSL-2-Enhanced, ABSL-3, or BSL-3 depending on the virus, type of research, the institutional risk assessment, and regulatory agency's requirements.[2073] SARS-CoV and H5N1 influenza virus are Biological Select agents and Toxins (BSAT), and no GoF virus included in this process is a Tier 1 BSAT. Although influenza, SARS-CoV, and MERS-CoV are not Tier 1 BSAT, security governance of Tier 1 pathogens was included because a Notice of Proposed Rule Making was issued about the elevation of laboratory-generated, mammalian-transmissible H5N1 influenza virus to Tier 1 status.

All of the data collected about the potential threats and security governance were used to assess the plausible threats facing laboratories that study or store GoF virus(s). These plausible threats serve as the most probable events that could lead to a loss of containment from a biosecurity incident. Therefore, they

[2073] Research Administrator Interviews.

were used to focus the quantitative analysis of local and widespread infections on those acts that are the most plausible in today's laboratory security environment.

16.2 Methodology

The GoF studies considered in this report remain restricted to research that achieves enhanced virus production, enhanced pathogenicity, transmission in mammals, and/or evasion of natural immunity or medical countermeasures in influenza, SARS -CoV, and MERS-CoV virus strains, consistent with the framework proposed by NSABB.[2074]

The biosecurity RA is divided into two chapters. The first chapter evaluates the consequences of plausible biosecurity risks posed by malicious actors and acts targeting laboratories in which GoF viruses are studied or stored. The risks posed by the independent replication of published GoF research by malicious actors is examined separately in the chapter on Biosecurity Risk of Information.

The assessment of "Malicious Acts Targeting a Laboratory" is grounded in knowledge about biosecurity procedures at US research institutions, biosecurity governance in the United States, and biological and conventional threats facing US research institutions; this assessment follows the methodology section.[2075] The malicious actors considered as part of this evaluation include a lone insider, a lone outsider, organized criminals, domestic terrorists and extremists, transnational terrorists, and foreign intelligence entities. Data was collected through analysis of open source material, which consisted of reviews of government documents, the Bureau of Labor Statistics workplace incident database, peer-reviewed journal articles, academic databases and working papers, mass media accounts, and public documents on and imagery of select laboratories. This effort, conducted at the unclassified level, was supplemented by interviews with biosafety and/or biosecurity officials and researchers at various laboratories around the country, and with members of the law enforcement and intelligence communities.

The assessment was conducted in four stages: 1) identification of possible threats, including the type of actor, type of deliberate security breach, and possible consequences of a successful breach (an assessment of the "offense"); 2) identification of the layered security measures employed at US research institutions to mitigate malicious actor risk and any challenges associated with the implementation of those measures (as assessment of the "defense"); 3) assessment of overall security risks using realistic scenarios that are based on the information collected as part of steps 1 and 2; 4) evaluation of the potential for a plausible threat to cause local or global outbreaks based on the epidemiological modeling of the consequences of such threats; and 5) comparison of possible pandemic consequences of plausible threats involving GoF viruses and non-GoF viruses.

The following subsections describe the methodology employed in conducting the offense assessment, the defense assessment, and the interviews.

16.2.1 Assessment of Malicious Actors

The offense assessment identifies possible threats, including the type of actor, type of deliberate security breach, and possible consequences associated with a successful breach.

[2074] Gryphon Scientific (2015) "Conducting Risk & Benefit Analysis of Gain of Function Research: Initial Draft Workplan"

[2075] An overview of bioterrorism risk assessment methodologies can be found in: Bruce K. Hope, Sarah Elrod, "Risk Assessment in Bioterrorism," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 543-547.

A threat matrix was created from the assessment of historical cases, current events, and discussions with members of the intelligence community, who provided helpful context and background about potential malicious actors and malicious acts to take into account. The threat matrix, which is described in the next section, provides the basis for evaluating historical and possible threats associated with research laboratories in the United States.

16.2.2 Historical Analysis

The first step in the offense assessment process involved a historical analysis of attacks against laboratories (Appendix V: Section 16.3), biocrimes committed by individuals (Appendix V: Section 16.4), and terrorist interest in biological warfare (Appendix V: Sections 16.5-16.9) In general, collection of historical incidents was restricted to the 25-years from 1990 to 2015. Information about different incidents (i.e., biocrimes, laboratory attacks, or terrorist interest) varied in quality before 1990. In addition, laboratory governance and security changed dramatically in the 1980s, 1990s, and 2000s suggesting little relevance of older laboratory attacks or biocrime incidents. However, incidents that occurred before 1990 were included if they provided an indication of actor motivation, interest and/or capability, or possible type of act. Information about historical incidents involving biocrimes from 1975 to 2015, laboratory attacks from 1990 to 2015 (two incidents included were from the 1980s), transnational terrorists from 1980 to 2015, and the domestic terrorist and extremists from the 1950s to 2015 was collected.[2076] In total, eighty-four generic malicious actor–malicious act pairings, and ninety-six malicious act–possible loss of containment pairings, were considered.

Each of the malicious actor-malicious act pairings were then analyzed and grouped into sections by malicious actor type in Appendix V, Section 16.7. For each of the pairings, available historical cases were identified in open source literature. For pairings with no historical precedent, the possibility of occurrence of a case and its potential consequences was considered based on malicious actor motivations and capability or similarity to historical incidents. This assessment was carried out by looking at the actor's potential motivation and capabilities to carry out the given act. Historical cases that shared some similarities with a particular event were summarized, if sufficient information was available, as these cases provided a snapshot of the motivation and capabilities of malicious actors in carrying out similar events. Care was taken to identify certain cases where incidents may have occurred but, because of their nature, may not be documented in open source reporting (such as potential covert entries conducted by foreign intelligence entities). The findings from Section 7.4 were summarized in graphical fashion by filling-in cells of the threat matrix to identify historical cases and hypotheticals.

A summary of possible malicious actors and acts is presented in Section 7.6.1. This section draws lessons from the historical cases and hypotheticals considered in Section 7.4 and Appendix V, Section16.2-16.15.9. but also considers significant changes that have occurred in malicious actor capabilities and/or opportunities or motivations that could alter identified historical trends. In sum, this section presents a short-list of malicious actors, acts, and consequences that deserve further attention. An analysis of plausible malicious actor/malicious act combinations based on evaluating the offense and defensive measures together are described in Section 7.6.

16.2.3 Identification of Malicious Actors, Acts, and Consequences

The malicious actors considered were:

- Lone outsider,

[2076] The two incidents involving laboratory attacks in the 1980s were theft of infected animals.

- Lone insider,
- Organized criminals,
- Domestic terrorists and extremists,
- Transnational terrorists including state-like terrorist groups, and
- Foreign intelligence entities.

The attack vectors considered were:

- Armed assault,
- Bomb or arson,
- Physical covert entry,
- Cyber covert entry,
- Theft of pathogens,
- Theft of equipment or materials,
- Sabotage, elicitation of information,
- Subversion of employee,
- Insertion of operative,
- Self-infection, and
- Reckless acts.

The potential consequences of such acts that might lead to a disease outbreak include the release of infected animals from/within a laboratory, the release of infected laboratory animals into the environment, the cross-contamination of laboratory animals, the deliberate exposure of a laboratory worker, and the removal of a pathogen sample from the laboratory. Three types of infections may result from outdoor release of pathogen: the deliberate infection of wild or domestic animals, the deliberate infection of laboratory workers, and/or the deliberate infection of members of the general public.

Other possible consequences include monetary or personal benefit by malicious actors. These consequences are not included in the security risk assessment because they do not directly affect human health. However, the consequence of personal benefit, rather than of exposure or release of an agent, aligns more closely with acts carried out by some malicious actors.

This threat matrix was used to identify probable security scenarios that was used to model the pandemic potential of an intentional release. Identifying these scenarios involved three distinct steps: 1) considering historical examples; 2) extrapolating possible actor/act/consequence combinations based on actor motivation and capability; and 3) analyzing the probable actor/act/consequences combinations (referred to as plausible threats) after overlaying current defensive measures onto the threat matrix. Threats motivated by financial or other personal gain were not assessed in the qualitative biosecurity risk assessment.

The parameters of the threat matrix is shown in Figure 16.1, below.

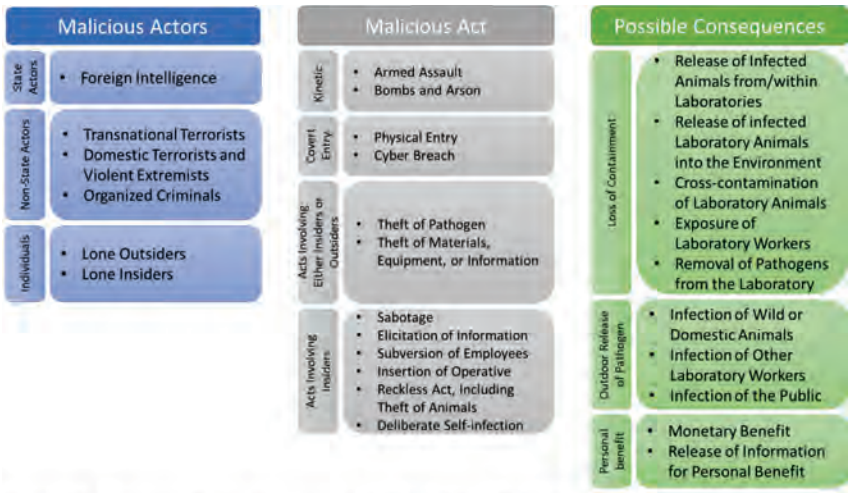

Figure 16.1. Threat matrix of malicious actors, malicious acts, and possible consequences.

16.2.4 Defense Assessment of Governance of Defensive Measures

The assessment of defensive measures, including governance, identifies the layered security measures employed at US research institutions, and inconsistencies and/or challenges associated with the implementation of those security measures. Laboratory defenses against malicious actors are derived from both biosafety and biosecurity oriented policies and practices, since safety-oriented measures restrict the operating environment and often provide security benefits. As such, safety measures that also provide security benefits was considered as part of the overall defense assessment.

The defense assessment begins with an overview of the tiered, agent-specific, and experiment-specific operating framework. This overview highlights what types of laboratory operating frameworks are currently approved to work with the GoF pathogens of interest to this report. Included in the assessment were the requirements and practices related to key aspects of malicious actor defense per the Statement of Work for this effort, namely: personnel training; personnel reliability; physical security; surveillance and monitoring; storage, inventory, and accountability processes; hazardous chemicals protocols; transfer, shipment, and chain-of-custody protocols; and emergency response protocols.

Sources used include federal laws, federal regulations, Executive Orders, international and domestic guidance documents, and official inspection reports released through the Freedom of Information Act process. The *US Code* and acts of Congress published in the *Federal Register* were used to retrieve laws.[2077] Federal regulations were retrieved for review through the Electronic Code of Federal Regulations.[2078] These sources were supplemented by peer-reviewed journal articles on implementation, news articles, and information derived from interviews.

[2077] U.S. Government Publishing Office http://www.gpo.gov/fdsys/browse/collection.action?collectionCode=PLAW
[2078] U.S. Government Publishing Office, "Electronic Code of Federal Regulations" www.ecfr.gov.

16.2.5 Interview Methodology

16.2.5.1 Intelligence and Law Enforcement Officials

Gryphon Scientific conducted a series of interviews with Federal intelligence and law enforcement officials to develop the final threat matrix and identify those threats that pose the greatest concern to US national security. The questions asked ranged from the types of actors that are thought to target biological laboratories to the types of methods that could breach physical and cyber security measures. The interview script is included in Appendix III: Section 14.9.

At the For Official Use Only level, the interviews provided valuable insight into the various malicious actor types, their motivations and capabilities, types of malicious acts, and types of consequences that should be included in the threat matrix.

The final threat matrix was used to map: 1) possible threats based on motivation and capability derived from information obtained from open source publications and the interviews conducted with intelligence and federal law enforcement officials; and 2) historical examples primarily based only on open source literature.

16.2.5.2 Research Institution Officials and Scientists

Gryphon Scientific reached out to seven research institutions that conduct Gain of Function studies as part of the biosecurity risk assessment process. Research was paused at six of these institutions.[2079] Scientists and officials were interviewed from a total of six research institutions, as one institution opted not to participate in the biosecurity interviews.

Also interviewed were principal investigators whose research was paused, students and staff in their laboratories, directors of institutional environmental health and safety, biosafety officials, campus police, and staff responsible for emergency response. For the five institutions that worked with select agents, the responsible officials and alternate responsible officials of the Biological Select Agents and Toxins Program were interviewed. At three institutions, the local FBI WMD Coordinators who serve as liaisons between federal law enforcement and research institution officials were interviewed. At one institution, the Vice President of Research, General Counsel, and Director of Human Resources spoke to project staff. The interview script is included in Appendix III: Section 14.12.

The majority of the biosecurity interviews were conducted concurrently with other team members conducting the RBA, which enabled a deeper understanding of certain measures that cross over between safety and security. Implementation of security and crossover safety measures at research institutions that support Gain of Function research are included in the research governance section (Appendix III, Section 14.11).

The results of these interviews contributed to the development of security case scenarios by providing greater understanding of how regulatory requirements are implemented at institutions that conduct GoF influenza, MERS-CoV, and SARS-CoV research. These institutions do not represent *all* research institutions that support infectious disease research, and specifically Biological Select Agents and Toxins (BSAT) regulated research. Despite this shortcoming, the case scenarios developed to evaluate the pandemic potential of malicious acts involving GoF research reflect accurately the safety and security

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[2079] Jocelyn Kaiser, "Moratorium on risky virology studies leaves work at 14 institutions in limbo," *Science Insider*, November 17, 2014, http://news.sciencemag.org/biology/2014/11/moratorium-risky-virology-studies-leaves-work-14-institutions-limbo.

conditions at the institution wherein this research is conducted. Therefore, the results are informative for all institutions under the moratorium.

Information about the primary threats facing research institutions at which the interview was conducted are included in the threat matrix (Section 7.4) and described briefly in the threat section (Appendix V- Section 16.2-16.9).

16.2.5.3 Interview Guide for the Biosecurity Risk Assessment

In-person interviews will be semi-structured to allow us to ask follow-up questions as necessary.

Interview Script for Intelligence and Law Enforcement Officials
Part 1: Capabilities and Motivations of Hostile Actors + Potential Hostile Act Modalities

- *Overarching Question: What is the risk that may ensue based on the successful targeting of a biolab facility in the US on the part of a malicious actor (i.e., target attractiveness)?*

- What are the various types of malicious actors that have posed or may pose a specific threat in this area and what is their demonstrated or postulated motivation for targeting a biolab facility?
  - What types of actors are known to have targeted laboratories or may find laboratories an attractive target to:
    - Cause an intentional on-site release of an agent,
    - Cause facility disruption or destruction,
    - Acquire information, agent, or expertise for malicious purposes? and
    - Can you provide specific examples of the above?

  - What types of actors have joined or would be most likely to join laboratories to build their own skills?

  - Are any types of actors likely to acquire a strain from a laboratory but NOT use them (use them in their own R&D programs or defensive programs).

  - Is the distribution of these actors equal throughout the world or more concentrated in one or more specific region(s), or one or more category(ies) of malicious actor?

- Are certain types of malicious actor threats and malicious act modalities more prevalent, more likely and/or more concerning than others? Why or why not?

- Would a successful hostile act against a biolab facility achieve the stated or postulated objectives of a given threat actor (see Threat Matrix)? More so than a hostile action against another type of target?

- Would you recommend any adjustments to our draft threat matrix (provided in advance) based on your knowledge and understanding of potential malicious actor threats to laboratory facilities? If so, what are specific things we should improve or change?

- How might malicious actors target and take action against a laboratory to gain access to materials or expertise relating to GOF research (i.e., tactics, techniques and procedures)?

- What specific capabilities are required to permit malicious actor access to or launch an attack against a facility?
  - Physical,
  - Cyber, and
  - Documentation.

- In what ways have actors tried to gain access to facilities, materials and expertise relating to advanced genetic engineering or, more specifically, GOF research?

- Can you recommend any additional studies, reports, analyses, real world case studies, etc. that would be important for us to consider in better understanding actor capability, access and motivation?

- Can you recommend anyone else who would be important for us to interview?

Information Risk Questions:

- Which actors, if any, are interested in the use of contagious agents in an attack? Does the possibility that the US has a relatively robust public health system to mitigate an outbreak and therefore many/most deaths may occur elsewhere figure into the calculus of these actors?

- Is influenza virus or MERS or SARS-CoV particularly of interest to any actor (compared to other deadly, contagious agents)?

- Has any substate actor shown any interest in manipulating a biological agent to make it more dangerous?

- Does any substate actor have the capability to manipulate a viral agent?

- How long is a substate actor willing/capable to work on developing an agent to execute an attack?

- Have any actors (state or substate) been known to insert operatives into a laboratory to gain knowledge or skills in particular techniques in the life sciences for the purposes of developing a weapon?

- Are any actors interested in agents that are countermeasure resistant?

- Does the publication in the scientific literature of *various* methods to modify a dangerous pathogen increase state/substate actor interest in attaining a biological agent or modifying a pathogen to make it more dangerous compared to the publication of just one route to modify a pathogen? Or is a terrorist who is interested in modifying an agent going to seek out means to do so from the literature, regardless of how many dual-use articles are published?

Interview Script for Environmental Health & Safety, Biosafety and Institutional Security Officials
Part 2: Gaps in Biosecurity policies, plans and implementation

- *Overarching Question: What is the probability of an incident arising from shortcomings or exploitation of vulnerabilities in the security of pathogens?*

- Do the current biolab security policy/regulatory environment and the implementation of the security requirements mandated therein adequately address the various types of potential malicious actor threats?

- In your opinion, are there specific gaps in policy or regulation (including staff awareness and training programs) or in the implementation thereof that represent an exploitable vulnerability? (please address the areas listed below)
  - Personnel reliability/security,
  - Physical/electronic access control,
  - Inventory/accountability processes,
  - Pathogen storage protocols,
  - Transfer, shipment, and chain-of-custody protocols,
  - Surveillance and monitoring,
  - Malicious actor detection,
  - Incident reporting, and
  - Emergency response protocols.

- What challenges do you face in implementing current federal regulations? How might these challenges affect facility vulnerability (increase, decrease, or no change)?

- Are there state and local laws that increase the vulnerability related to unauthorized individuals gaining access to information, counter federal regulations, or impose barriers to implementation of federal regulations?

- What state and local laws decrease biolab facility vulnerability or otherwise support federal regulations?

- Have you ever experienced a malicious actor threat to or act against your facility?

- Are representative security plans and training/awareness programs for high containment facilities in alignment with governing policies/regulations and best practices, and, if so, do they adequately address the threat? Are there specific gaps or concerns?

- In your opinion, if gaps exist in terms of policy or regulation or in the implementation thereof, what are your recommendations to remedy them?

- In addition to policy and/or regulatory requirements, are there any best-practices for biolab security (in use domestically or internationally) that you would recommend?

- To what degree does your institution interact with the local FBI WMD Coordinator to stay ahead of potential threats and to inform them of potential problems?

- Can you recommend any additional studies, reports, analyses, real world case studies etc. that would be important for us to consider?

- Can you recommend anyone else who would be important for us to interview?

Interview Script for Researchers

- *Overarching Question: What is the probability of misuse or theft arising from authorized laboratory staff?*
- What processes/protocols exist in the laboratory and within the institution to prevent misuse of research, theft of agent, or malicious use of an agent? Are there specific gaps or concerns?
- What types of biological security training do laboratory staff receive? Are there specific gaps or concerns?
- What processes exist for researchers to report suspicious or unusual events or actions? Are there specific gaps or concerns?
- What processes exist to interact with relevant institutional officials to identify and reduce security risks associated with your research? Are there specific gaps or concerns?
- Do laboratory staff consider security (i.e., misuse of research or theft) a high priority concern?
- Can you recommend any additional studies, reports, analyses, real world case studies etc. that would be important for us to consider?
- Can you recommend anyone else who would be important for us to interview?

16.2.6 Semi-Quantitative Analysis of Plausible Threats

From the assessment of the offense and the defense, "relatively high-risk" threat scenarios were created that match the motivations and capabilities of the malicious actors, the malicious acts they may attempt and, in light of the defenses arrayed against them, the outcomes these events are likely to have. Simply put, combinations of actor and act were compared against the defense to choose a set that are most likely to have a bad outcome out of all other possibilities. From this comparison, qualitative statements can be made on the frequency that these acts are likely to be attempted (based on the historical record, the motivation of malicious actors and the overall activity level of malicious actors) and how likely they are to be successful.

16.2.6.1 Qualitative Analysis of Plausible Threats

Narrowing down the universe of possible threats (Section 7.4, Appendix V Section 16.2-16.9) to probable threats associated with US laboratories conducting GoF influenza, SARS-CoV, and MERS-CoV research involves systematic evaluation of the ability of implemented security measures to prevent malicious actors from accessing laboratory materials, animals, or pathogens and carrying out malicious acts at the laboratories. This analysis consists of two steps: 1) assessment of malicious actor intent, capability, and ability to access high containment research laboratories given existing security measures and in light of historical occurrences and expressed interest; and 2) evaluation of the likelihood of success of malicious acts in the presence of existing security measures and of a successful act resulting in virus escape (i.e., loss of containment). The qualitative assessment of plausible threats is based on an analysis of historical examples and motivation/capability of malicious actors. This approach eliminates completely implausible scenarios, such as the use of drones to deliver packages inside a laboratory without detection, from the analysis.

Figure 16.2 depicts the process diagram for analyzing plausible threats based on offense and defensive measures.

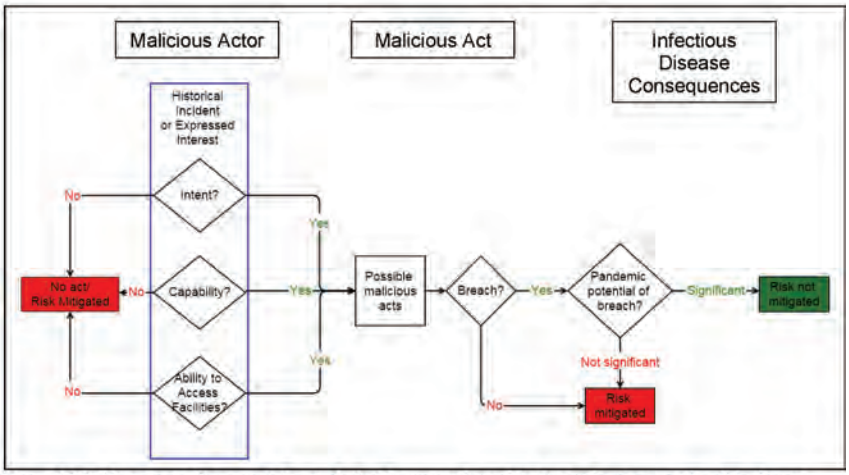

Figure 16.2. The process diagram is the analytical framework used in the study to assess plausible threats based on the offensive and defensive measures. Red indicated low or no risk. Green indicates risk.

The first step of this analysis involves assessing the malicious actor's *intent* to develop and use biological agents as weapons and/or to breach a research laboratory to acquire the pathogen, material, equipment or animal; *capability* that a malicious actor could commit a malicious act, including acquisition of a pathogen; and *ability* to gain access to a high containment, research laboratory and its contents, regardless of whether the laboratory is the source of the agent or the target of an attack. In assessing the intent, capability, and ability of the different malicious actors listed in the threat matrix (Appendix V Section 16.1), the relative success of an insider compared to an outsider was assessed. This assessment is based on analysis of the historical incidents and the evaluation of malicious actor motivations and capabilities.

An actor needs to have sufficient capability to commit an act. Capabilities includes specialized skill, expertise, access to materials, and support. Capabilities of individuals who 1) subvert or elicit an insider, 2) are an outsider trying to commit a malicious act, and 3) an insider intent on commit malicious acts will be evaluated separately. Defensive measures preventing or introducing barriers to capability are incorporated in the analysis.

The second step involves assessing the likelihood that a particular malicious act could be carried out successfully given currently implemented security measures and the likelihood that a successful act could cause a virus escape (i.e., loss of containment). This analysis is based on federal requirements for security of Biological Select Agents and Toxins and implementation of crossover biosafety and biosecurity measures at the research institutions that project staff visited as part of this project. Because no systematic analysis has been conducted to identify the state of safety and security measures at all high containment facilities and BSAT facilities, our analysis does presume that the institutions visited do not represent all institutions with high containment facilities. However, research was paused at five of the six institutions

project staff visited as part of the GoF deliberative process, which is roughly one-third of the 14 institutions that received "stop work" orders from the National Institutes of Health.

Plausible threats that could be faced by US research institutions that conduct GoF research with influenza, SARS-CoV, and MERS-CoV viruses were produced from this analysis. These threats were grouped into three broad categories: 1) overt acts; 2) covert acts exposing members of the public; and 3) covert acts exposing laboratory workers. Overt acts involve incidents, such as bombs or active shooters that would trigger emergency personnel to respond. Covert acts involve acts that are not carried out openly and about which emergency personnel may not be aware. Covert acts are divided into those exposing the public and those exposing laboratory workers to capture differences in health monitoring and familiarity of the viruses in the research laboratory and the symptoms they cause in infected individuals between each group of people. These categories of plausible threats will be analyzed using epidemiological modeling as described for the Biosafety RA.

16.2.6.2 Semi-Quantitative Epidemiological Modeling of Security Risk Scenarios.

For each of these "relatively high-risk" scenarios that result in a loss of containment, the possible outcomes were modelled by linking to results from the Quantitative Biosafety Risk Assessment (described above). For example, if a malicious act results in the accidental or intentional release of an aerosol, calculations performed in the Biosafety RA can help determine what the consequences of that release would likely be for an aerosol that initially infected any number of people. Quantitative analysis will be conducted using different numbers of people exposed: one infected individual, ten or less infected individuals, or greater than ten infected individuals. Analysis of the plausible threats is qualitative while the consequences of the threat can be quantitatively assessed based on the epidemiological models developed for the biosafety risk assessment.

16.3 Definitions of Terms Used in the Threat Matrix

16.3.1 Malicious Actors

The threat matrix includes seven actors: lone outsiders, lone insiders, organized criminals, domestic terrorists and violent extremists, transnational terrorists, state-like transnational terrorists, and foreign intelligence entities.

The US Code of Federal Regulations defines terrorism as, "the unlawful use of force and violence against persons or property to intimidate or coerce a government, the civilian population, or any segment thereof, in furtherance of political or social objectives."[2080,2081] This definition was used in our study to distinguish terrorists from criminals.

16.3.1.1.1 Lone Outsiders

Lone outsiders are unaffiliated individuals who have an interest in attacking or gaining access to research facilities, materials, agents, experimental protocols, or results. They are not affiliated with any particular group. However, they may aspire to become members or may simply act in independent support of an existing group. They are not a student or employee of a research facility. The motivations and capabilities of these actors vary greatly.

[2080] Federal Bureau of Investigation (FBI), "Definitions of Terrorism in the U.S. Code," https://www.fbi.gov/about-us/investigate/terrorism/terrorism-definition. Accessed on July 17, 2015.

[2081] Federal Bureau of Investigation (FBI), "Terrorism 2002-2005," https://www.fbi.gov/stats-services/publications/terrorism-2002-2005. Accessed on July 17, 2015.

16.3.1.1.2 Lone Insiders

Lone insiders are unaffiliated individuals who work in a research facility and have interest in using research materials or agents to cause harm. These actors are of particular interest because many have the scientific training to manipulate biological agents. Various research facility support staff have access to research materials and agents, but they do not necessarily have the scientific capabilities required to conduct experimental protocols. Similar to the variability of capabilities, the motivations of these actors varies greatly, ranging from ideological radicalization to emotionally-motivated behavior.

16.3.1.2 Organized Criminals

Organized criminals are defined here using the FBI's definition of organized crime: "any group having some manner of a formalized structure and whose primary objective is to obtain money through illegal activities."[2082]

16.3.1.3 Domestic Terrorists and Violent Extremists

Domestic terrorists and violent extremists include groups and their members who vandalize, attack, or otherwise harm facilities and individuals to make a political statement, protest for a cause, or "correct" a real or perceived wrong. These groups include violent extremists, such as some animal rights or eco-radical groups, and cults that use violence to achieve their goals. Some individuals who are affiliated with extremist organizations have tried to acquire biological agents from culture repositories.

16.3.1.4 Transnational Terrorists, Non-State Actors

Transnational terrorists, non-state actors refer to non-state groups that operate across national borders and have similar ideological and/or political interests. This category encompasses those actors that are attempting to control and govern territory and use tactics that do not conform to international norms for war and nation-building. A prominent example of such an actor is the Islamic State of Iraq and the Levant (ISIL). These groups use violence to achieve their political goals and to attack other nations or groups that they perceive as enemies.

US-based individuals radicalized by transnational groups are considered within the scope of this entry.

16.3.1.5 Foreign Intelligence Entities

Foreign intelligence agencies are a branch of a foreign government or of its armed forces tasked with conducting espionage against other countries.[2083] The term "Foreign intelligence entities" refers to individuals working for nation states to collect information about research efforts.

16.3.2 Malicious Acts

The threat matrix includes 12 different malicious acts, which are divided into four categories: 1) kinetic attacks including armed assault and bombs or arson; 2) covert entry including physical entry or cyber breach; 3) theft of materials or pathogens by an outsider or insider; and 4) acts involving insiders, which

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[2082] Federal Bureau of Investigation (FBI), "Glossary of Terms," http://www.fbi.gov/about-us/investigate/organizedcrime/glossary. Accessed on July 13, 2015.

[2083] For related definitions, see for instance the old Foreign Intelligence Surveillance Act of 1978. "50 U.S. Code Chapter 36- Foreign Intelligence Surveillance," U.S. Code Title 50 Chapter 36, Subchapter I- Electronic Surveillance (§§ 1801-1812), https://www.law.cornell.edu/uscode/text/50/1801. Accessed August 11, 2015.

includes sabotage, elicitation, subversion of employee, insertion of an operative, reckless intentional act, and self-infection.

16.3.2.1 Kinetic Attacks

Armed assault

An armed assault refers to the use of firearms to harm individuals who work at research facilities or forcibly gain access to facilities.

Bombs or arson

Bombs refer to any type of explosive used to attack individuals or breach laboratories. These explosives can be homemade from chemicals, military devices such as grenades, or commercial devices. Arson refers to the deliberate starting of a fire at a facility. This fire can be caused by an incendiary device or other explosives.

16.3.2.2 Covert Entry

Physical entry

Covert physical entry of a facility refers to the physical access of a research facility or laboratory by an unauthorized individual without detection.

Cyber breach[208A]

Cyber breach refers to the non-physical and unauthorized access of, and subsequent interaction with, a computer or other electronic device linked in some manner to laboratory research. It includes hacking, denial of service attacks, insertion of a computer viruses, and other computer-based breaches to access facility engineering systems, facility or laboratory computers, or human resource information. These breaches could disrupt operations, facilitate theft of information, or tamper with engineering controls.

Care is taken to distinguish between attacks against devices owned by the researchers themselves, Internet-connected devices within a laboratory, and so-called "air-gapped" network(s) within the laboratory that are isolated from the Internet. Unauthorized access can be gained remotely in different ways, for instance through malicious webpages (e.g., watering hole attacks), email attachments (e.g., spear phishing attacks), or the insertion of malicious programs on USBs or CD-ROMs subsequently inserted into the target network. These breaches could be used to steal information, facilitate a break-in, or potentially to tamper with engineering controls.

Cyber breaches are included in the matrix because malicious actors have attacked computer systems. However, they will not be analyzed because they likely do not result in direct human health consequences.

[208A] Whether cyber breaches of a laboratory could directly lead to human health consequences through sabotage is outside the scope of this assessment. The Department of Defense (DOD)'s Defense Science Board Task Force considered the potential threat of cyber-sabotage in their May 2009 assessment of DoD laboratory security, and recommended that an in-depth study be conducted to determine the potential cyber threat against U.S. laboratories. As discussed in their report, a proper assessment of potential cyber-sabotage threats against a U.S. biological laboratory would necessitate full on-site access to a U.S. laboratory to "identify actual or potential access" to its IT infrastructure. Office of the Under Secretary of Defense For Acquisition, Technology, and Logistics, Defense Science Board, "Report of the Defense Science Board Task Force on Department of Defense Biological Safety and Security Program," May 2009, p. xii, 18-19, 41, http://www.acq.osd.mil/dsb/reports/ADA499977.pdf. Accessed September 27, 2015.

16.3.2.3 Acts Caused by Either Insiders or Outsiders:

Theft of pathogen

Theft of pathogen refers to the unauthorized removal of a pathogen from long-term storage or from experimental samples.

Theft of materials, equipment, or information

Theft of materials refers to the unauthorized removal of research reagents, chemicals, equipment, experimental kits, research notes, information, or other items from a research laboratory or facility.

16.3.2.4 Acts Involving Insiders:

Sabotage

Sabotage refers to the deliberate destruction of laboratory equipment, experiments, stocks, or results. Often, these acts are driven by personal gain, revenge, competition, or other personal motivations, rather than a desire to cause a deliberate release of agent. However, certain acts of sabotage have the potential to cause a loss of containment, regardless of the actual intent of the malicious actor. For example, mixing animals from different experiments or mixing infected and uninfected animals could result in cross-contamination of research animals.

Elicitation

Elicitation refers to the manipulation of an individual to gain information about the research and facility. Desired information could involve research activities, research animal housing, research results, pathogen storage locations and procedures, and facility and laboratory operations, procedures, and security measures.

Subversion of an employee

Subversion of an employee refers to an actor actively working against an employee to gain physical access to research facilities and agents, steal pathogens, or acquire information about the research or facilities.

Insertion of an operative

Insertion of an operative refers to a member of an organization, group, or nation that joins a research laboratory or facility as a student, employee, or authorized visitor. The individual is not known to be a malicious actor or to be affiliated with a malicious organization by the institution they are infiltrating. An operative can insert themselves to gain access to information, pathogens, or laboratory individuals and facilities. This individual also may join a laboratory to build his or her skills in carrying out a particular set of experiments, gain access to key scientists, or provide themselves with an opportunity to acquire reagents, agent, or equipment.

Reckless intentional act

A reckless intentional act refers to a situation wherein a deliberate act accidentally results in loss of containment. For example, the deliberate release of infected research animals by animal rights groups would result in release of agent into the environment. In the discussion about historical incidents, theft of

animals is categorized as a reckless act. However, we separate theft of animals in the qualitative plausible threat analysis.

Deliberate self-infection

Self-infection refers to an individual who deliberately infects himself/herself. This action does not presuppose that the intention for infecting oneself is to infect others; self-infection may be done in an attempt to commit suicide, cause self-harm, or conduct an unauthorized experiment using him/herself.

16.3.3 Possible Consequences of Successful Act

The threat matrix includes eight possible consequences resulting in loss of containment, which are divided into two categories: 1) loss of containment resulting in release of infected animals from and within laboratories, release of infected animals into the environment, cross-contamination of laboratory animals, exposure of laboratory workers, and removal of a pathogen from the laboratory; and 2) outdoor release of pathogen resulting in infection of wild or domestic animals, infection of other laboratory workers, or infection of the public.

16.3.3.1 Loss of Primary Containment:

Loss of Containment includes intentional or accidental release of infected research animals or agent from the laboratory. It does not refer to outdoor release such as release of agent directly from a facility into the environment as a liquid or aerosol.

Release of infected animals from and within laboratories

Release of infected animals from and within laboratories refers to the escape of research animals from their housing, hoods, or other spaces within individual rooms or between rooms of the containment laboratory. This consequence does not presuppose that the animal leaves the containment facility.

Release of infected animals into the environment

Release of infected animals into the environment refers to the facilitated escape of research animals outside of the containment facility and into biosafety level (BSL) 1 or 2 laboratories. Release into BSL-1 or BSL-2 laboratories may lead to release of the pathogen outside the building and into the environment.

Cross-contamination of laboratory animals

Cross-contamination of laboratory animals refers to the mixing of research animals from different experiments or the mixing of infected research animals with uninfected research animals. Often, animals in experiments are housed separately from other experimental animals or unused animals. Experimental animals are housed in vivariums. Special facilities exist for housing animals infected with Biological Select Agents and Toxins (BSAT).

Exposure of laboratory workers

Exposure of laboratory workers refers to at least one laboratory worker that might be infected by a pathogen through a needle stick, tear in personal protective equipment, animal bites or scratches, or other means of exposing a worker to a pathogen.

Removal of a pathogen from the laboratory

Removal of a pathogen from the laboratory refers to the physical removal of the pathogen from experimental samples, infected animals, or stored inventory.

16.3.3.2 Outdoor Release of Pathogen:

Outdoor release of pathogen includes liquid or aerosol release of a pathogen into the environment or neighboring community. The neighboring community includes laboratory workers who do not work with the pathogen and the broader public. Examples of release into the environment include efflux of aerosolized agent into the atmosphere caused by reversal of air handling systems or removal of HEPA filters and release of liquid agent into the soil from sabotaged pipes or disposal measures.

Infection of wild or domestic animals

Infection of wild or domestic animals refers to infection of household animals, livestock, or wild animals because of liquid or aerosol release of pathogen into the environment. This event could include release of infected research animals into the environment. In addition, it does include environmental release of agent from animal carcasses that have not been properly disposed of.

Infection of other laboratory workers

Infection of other laboratory workers refers to the infection of individuals who do not work directly with the pathogen, but do work in the same facility or on the same research campus. Infection may occur through aerosol from contaminated equipment, improper decontamination or fixation of samples, or residue on clothing or other materials that came in direct contact with the agent.

Infection of the public

Infection of the public refers to a release in which at least one individual of the general public is infected with a laboratory generated or adapted pathogen. Such infections could be caused by the deliberate release of pathogens into the atmosphere, ground water, or soil in close proximity to neighborhoods, commercial spaces, and parks and other outdoor spaces.

16.3.3.3 Personal Benefit

Other possible consequences include monetary or personal benefit by malicious actors. These consequences are not included in the security risk assessment because they do not directly affect human health. However, the consequence of personal benefit, rather than of exposure or release of an agent, aligns more closely with acts carried out by some malicious actors.

Monetary benefit

Monetary gain refers to financial benefit from selling stolen items on the black market or developing a commercial product using stolen samples or data.

Release of information for personal benefit

Release of information for personal gain refers to individual or group benefit including getting ahead of competitors, revenge, financial gain, and reputational gain.

16.4 Analysis of Malicious Actor Capabilities and Motivations

This section provides an overview of the motivations and capabilities of the malicious actors considered

16.4.1 Lone Outsiders

16.4.1.1 Motivations

Lone outsiders could be motivated to carry out a malicious act by ideology, by personal grievances, for personal gain, or by a combination thereof. A malicious act may also be the work of a disturbed individual outsider, and, therefore, lack a rational, pre-mediated motive.

Examples of potential ideological opposition leading to a lone outsider carrying out a malicious act against a laboratory include: jihadist ideology, radical animal rights beliefs, radical environmental beliefs, radical anti-genetic engineering or anti-modernity ideologies, or anarchism, more broadly. Personal grievances could be directed against one or more individuals working at the laboratory, for instance an individual that rejected the lone outsider's candidacy to work at the laboratory, or spurned a romantic approach. Personal grievances could also be generated over the laboratory's presence in the locale where the individual lives, generating a radical "not-in-my-backyard" response. Finally, the use of illegal drugs, the misuse of legal drugs, or mental health issues may lead to an irrational, unpremeditated, malicious acts.

Since the potential motives behind a lone outsider act are extremely varied, the willingness of a lone outsider to risk death or capture in an attack is unpredictable. Similarly, a lone outsider's willingness to make ill or kill is highly unpredictable.

16.4.1.2 Capabilities

Lone outsiders can have, and have exhibited, a wide range of capabilities. Whilst rare, a few malicious lone outsiders, such as Ted Kaczynski (the "Unabomber"), Eric Robert Rudolph, and Muharem Kurbegovic (the "Alphabet bomber"), have been well-versed in bomb-making.[2085,2086] Others, such as Larry C. Ford (who was found to have possessed dangerous pathogens after his suicide), had professional experience handling dangerous pathogens.[2087] On average, lone outsiders are "often ineffectual," in the sense that their violent plots often fail to produce casualties.[2088] When lone actors do successfully commit direct violence, it is often through the use of firearms and sometimes explosives. For instance, scholar Ramon Spaaij's study of 88 cases of successful lone actor terrorism in the 1968 – 2010 period showed that 43% of cases involved the use of firearms and 28% involved the use of explosives.[2089] Lone outsiders

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2085 Jeffrey D. Simon. "The Alphabet Bomber (1974)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan Tucker (Cambridge: The MIT Press, 2001), 85-86.

2086 Federal Bureau of Investigation (FBI), "The Pursuit and Capture of Eric Rudolph: Part 1 of an Interview with FBI Exec Chris Swecker," May 2005, https://www.fbi.gov/news/stories/2005/may/swecker_051605.

2087 Jo Thomas, "California Doctor's Suicide Leaves Many Troubling Mysteries Unsolved," *The New York Times*, November 3, 2002, p. 1, http://www.nytimes.com/2002/11/03/us/california-doctor-s-suicide-leaves-many-troubling-mysteries-unsolved.html?pagewanted=1.

2088 The conclusion was stated in general for lone actors, but is clearly applicable to the subset composed of lone outsiders. Borum R, Fein R, Vossekuil B (2012) "Dimensional Approach to Analyzing Lone Offender Terrorism." *Aggression and Violent Behavior* 17, no. 5: 390.

2089 Restricting totals to U.S. cases sees an increase in the use of firearms.
Spaaij R (2012) *Understanding Lone Wolf Terrorism* Melbourne. Springer.
For a discussion of this study, also see: Paul Gill, *Lone-Actor Terrorists: A Behavioral Analysis* (Abingdon: Routledge, 2015), p. 16-17.

would most likely not have access to heavy weapons, such as rocket propelled grenade launchers, that could be used to breach containment walls.[2090]

16.4.2 Lone Insiders

16.4.2.1 Motivations

As with lone outsiders, lone insiders could be motivated to carry out a malicious act for ideological, personal, or financial reasons, or carry out unpremeditated and irrational acts as a result of mental health issues, legal drug abuse, or illegal drug use.[2091] A lone insider carrying out a malicious act against a laboratory may involve radicalization in sympathy to a jihadist ideology. Hypothetically, the possibility exists that a sudden disgust with one's research could lead one to turn to radical animal rights beliefs, radical environmental beliefs, radical anti-genetic engineering or anti-modernity ideologies, or anarchism; however, no such case was uncovered in open source reporting. Personal grievances against one or more individuals working at the laboratory, such as those potentially developed as a result of poor work relations, could drive an individual to engage in violent acts. Finally, the abuse of legal drugs, the use of illegal drugs, sudden emotional trauma, or mental health issues may lead to an irrational, unpremeditated, malicious act.

A lone insider's willingness to risk death and willingness to kill is unpredictable.

Unlike lone outsiders, a lone insider potentially will be subject to periodic personnel surety screening, ranging from simple checks for the use of illegal drugs to personnel reliability program and related background and criminal history checks, depending on the nature of their work and the type of facility in question. Although laboratory workers who work with Tier 1 Select Agents are screened and evaluated periodically, the potential that a worker becomes radicalized, disgruntled, or disturbed after hiring and between evaluations, cannot be discounted.[2092] Moreover, a worker could manage to mask their radical beliefs or mental health issues during initial screening.[2093] Finally, a lone insider may not be part of the universe of formally-screened personnel, if they are not working with Select Agents. As such, although personnel reliability programs are a first barrier against lone insider malicious acts, they are not a panacea for detection of this type of threat actor.[2094]

16.4.2.2 Capabilities

Given that a lone insider would be working at a laboratory, they are more likely than lone outsiders to have legitimate access to pathogens, research materials, and dangerous waste. Lone insiders are also much more likely to have training on the safe handling and movement of pathogens than a malicious lone outsider. Moreover, since a lone insider has some degree of inside access, attacks can be more complex than those launched by lone outsiders– for instance through the prepositioning of supplies within the

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2090 Although a lone outsider is unlikely to have the resources to obtain heavy weapons, this possibility cannot be entirely discounted, as such arms have fallen into the hands of organized groups in the U.S.in the past. For instance, law enforcement officials uncovered an Army light antitank weapon during their raid of the compound maintained by the "The Covenant, the Sword, and the Arm of the Lord" group. Jessica Eve Stern, "The Covenant, the Sword, and the Arm of the Lord (1985)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan Tucker (Cambridge: The MIT Press, 2001), p. 150.

2091 Biringer B, et al (2007) *Security Risk Assessment and Management: a Professional Guide for Protecting Buildings and Infrastructures* Hoboken: John Wiley & Sons.

2092 Bunn M, Sagan S (2014) *A Worst Practices Guide to Insider Threats: Lessons from Past Mistakes* Cambridge: American Academy of Arts and Sciences.

2093 AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 7.

2094 Bunn M, Sagan S (2014) *A Worst Practices Guide to Insider Threats: Lessons from Past Mistakes* Cambridge: American Academy of Arts and Sciences.

laboratory or through the exploitation of an internal safety system (such as a fire alarm). Finally, since a lone insider has some degree of inside access, they are far more likely to succeed in stealing pathogens or materials.

16.4.3 Organized Criminals

16.4.3.1 Motivations

Organized criminals seek to make a profit, for instance through the theft of equipment for sale on the black market or through extortion and racketeering. Criminal organizations might consider stealing and selling pathogens to terrorists, although no such historical examples are recorded in open sources. The profitability and market value of pathogens on the black market is not known.

16.4.3.2 Capabilities

Study of crimes against high-profile targets, which can be used as a proxy for crimes against laboratories, show that the capabilities deployed will be commensurate with the expected payoff.[2095] That is, organized criminals can hire or coerce highly qualified individuals and bring sophisticated equipment and weapons to bear for high-value activities (e.g., coercing engineers to establish a dedicated radio network in support of narcotics trafficking activities).[2096,2097] The ability of an organized criminal group to recruit individuals willing to risk death in an operation is proportional to its revenue. For instance, the wealthy drug cartels can draw from numerous individuals willing to kill and risk death for the organization, but a small-time thievery ring could not.[2098,2099] The likelihood that organized criminals have advanced scientific skill an access to pathogens is low.

16.4.4 Domestic Terrorists and Extremists

16.4.4.1 Motivations

Domestic terrorists and extremists are motivated by a number of ideological doctrines and political causes. The FBI categorizes malicious groups into far-left groups and far-right groups, although a domestic violent millenarian cult or home-grown Jihadi group unaffiliated with a transnational group might arise in the future.[2100] As demonstrated by the list of attacks against laboratories contained in Appendix V: Section 16.1, far-right groups have as of yet not attacked US laboratories, whilst far-left groups have routinely targeted US laboratories. The willingness of group members to harm or kill individuals also varies across these various types of terrorist and extremist groups, as explained below.

Far-left groups are currently mostly motivated by radical animal rights and radical ecological beliefs. These ideologies directly justify attacks against laboratories, and these groups are responsible for several breaches at US labs. Animal rights extremist groups such as the Animal Liberation Front (ALF), and some eco-radical groups like the Earth Liberation Front (ELF), explicitly forbid the killing of individuals,

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2095 Reinstedt RN, Westbury J (1980) "Major Crimes as Analogs to Potential Threats to Nuclear Facilities and Programs," RAND Note N-1498-SL, prepared for Sandia laboratories.

2096 "Mexico navy smashes Zetas cartel communications network," *BBC News*, September 8, 2011, http://www.bbc.com/news/world-latin-america-14846866.

2097 Beckhusen R (2012) "Mexican Cartels Enslave Engineers to Build Radio Network," *Wired* http://www.wired.com/2012/11/zeta-radio/.

2098 See for example: Jo Tuckman, "Mexican officials: 43 killed in major offensive against drug cartel," *The Guardian*, May 22, 2015, http://www.theguardian.com/world/2015/may/22/mexico-firefight-drug-cartel-region

2099 Cook C (2007) "Mexico's Drug Cartels," CRS Report for Congress http://fpp.fas.org/sgp/crs/row/RL34215.pdf.

2100 Ibid.

and members have so far adhered to this principle.[2101] Therefore, while ALF and ELF are motivated to target laboratories, they are not motivated to steal pathogens to harm others. This restraint is not universal across all far-left groups, as exemplified by the now defunct, very small, eco-radical group R.I.S.E. who sought to use pathogens to cause mass casualties (see Appendix V: Section 16.5). Eco-radical groups who are willing to kill scientists, using firearms and bombs, recently have emerged in Latin America and Europe.[2102,2103] In addition to the groups' vigorous propaganda against synthetic biology, they have targeted individuals working in the nanotechnology and nuclear sectors instead of high containment laboratories.[2104] In addition, they have not targeted US institutions or researchers.[2105]

In general, far-right groups are currently motivated by anti-government beliefs, radical religious beliefs associated with the Christian Identity movement, and racial supremacist notions.[2106,2107,2108] With the possible exception of federal laboratories, far-right group ideology currently does not promote attacks against laboratories. No reports in open sources describe a far-right group as having targeted a US biological laboratory. However, a radicalized researcher at a laboratory may attempt to smuggle out pathogens out for use against other targets. A select few far-right groups have shown some interest in biological weapons through their perpetration of biological weapons hoaxes (summarized in Appendix V: Section 16.6), but no group has so far displayed any actual biological weapons capability. For instance, a defunct group called the "Counter Holocaust Lobbyists of Hillel" sent agar and *B. cereus* in a petri dish to a Jewish organization and claimed the petri dish held *B. anthracis*, *Y. pestis*, or a chemical warfare agent as part of an apparent hoax.[2109] In general, far-right groups are likely to resort to violence and have carried out mass killings.[2110]

Domestic jihadi groups not affiliated or commanded by transnational groups or domestic violent millenarian cults have so far not emerged. Should such a group form, they are expected to favor mass casualties, as with transnational terrorist groups attacking US targets.[2111]

16.4.4.2 Capabilities

Capabilities vary across groups, mostly depending on the group's motivation and end goals. Averaging over a large number of past cases, when domestic and transnational terrorist groups commit acts of

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2101 Ackerman G (2003) "Beyond Arson? A Threat Assessment of the Earth Liberation Front," *Terrorism and Political Violence* 15, no. 4: 143-170.

2102 Phillips L (2012) "Anarchists attack science," *Nature (News)* 485, no. 561 http://www.nature.com/news/anarchists-attack-science-1.10729.

2103 Corral G (2011) "Stand up against the anti-technology terrorists," *Nature (News)* 476, no. 373, http://www.nature.com/news/2011/110822/full/476373a.html.

2104 Ibid.

2105 Ibid.

2106 Federal Bureau of Investigation (FBI), "Domestic Threat: White Supremacy Extremism," May 22, 2012, https://www.fbi.gov/news/stories/2012/may/extremism_052212/extremism_052212.

2107 Federal Bureau of Investigation (FBI), "The Terrorist Threat," February 6, 2002, https://www.fbi.gov/news/testimony/the-terrorist-threat-confronting-the-united-states.

2108 For a description of Christian Identity and far-right group ideologies, and an example of a far-right group, see: Jessica Eve Stern, "The Covenant, the Sword, and the Arm of the Lord (1985)," p. 141-144.

2109 Carus W (1996) *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 110-111; The B'nai B'rith International Jewish Monthly, Volume 111: 67, https://books.google.com/books?id=V--3AAAAIAAJ&q=anthracis+Yersinia+Counter+Holocaust+Lobbyists+of+Hillel&dq=anthracis+Yersinia+Counter+Holocaust+Lobbyists+of+Hillel&hl=en&sa=X&ved=0CC8Q6AEwA2oVChMI98TMwLKIxgIVOEaMCh0gNAC0.

2110 Shane S (2015) "Homegrown Extremists Tied to Deadlier Toll Than Jihadists in U.S. Since 9/11," *The New York Times*, http://www.nytimes.com/2015/06/25/us/tally-of-attacks-in-us-challenges-perceptions-of-top-terror-threat.html.

2111 For a discussion of the potential BW threat from domestic millenarian cults, see: Gary Ackerman, Markus Binder (for START), "Anatomizing the Behavior of Chemical and Biological Non-State Adversaries," PASCC Semi-Annual Workshop on Strategic Stability and WMD, Washington, U.S.A., December 5, 2014, p. 11, http://csis.org/files/attachments/141205_Ackerman_Slides_0.pdf.

violence, they frequently rely on the use of explosives (65-75% of cases).[2112,2113] Raids against far-right group safe houses have previously uncovered large amounts of weapons and explosives, demonstrating that domestic terrorists and extremists have access to the requisite weapons and equipment to carry out such attacks.[2114] Yet, the review of malicious acts against US laboratories presented in Appendix V: Section 16.1 reveals no bombings or armed assaults launched against US laboratories by far-right groups. Far-left groups have relied on night-time break-ins, often followed by arson using incendiary devices (Appendix V: Section 16.1). These groups have mostly eschewed the use of firearms, although some members have been known to own guns.[2115,2116] In sum, our efforts have not identified any attacks on US laboratories by a domestic terrorist or extremist group that were intended to produce casualties. The current non-use of firearms and explosives against US laboratories is not due to a lack of capabilities, but rather of motivation.

Several domestic extremist groups, both far-left and far-right, operate as decentralized cells. For example, the ALF and ELF operate through small isolated cells, which then publicize actions through pro-group outlets in the name of the overall organization.[2117] This organizational structure reduces the capabilities the group(s) can bring to bear against a single target, in return for greater resilience to law enforcement actions.[2118]

16.4.5 Transnational Terrorist Groups, including State-like Terrorist Groups

16.4.5.1 Motivations

The total number of terrorist groups targeting US citizens is low compared to the overall number of foreign terrorist groups currently in operation worldwide. One study found that a total of 395 terrorist organizations were active in the 1998 – 2005 period, where "active" was defined as having committed at least one attack in the given time period.[2119] In contrast, only 59 Designated Foreign Terrorist Organizations are currently listed on the official US list of active foreign terrorist organizations maintained by the US Department of State.[2120]

In general, modern transnational terrorist groups targeting the United States often are motivated by extremist religious ideology.[2121] These groups have tended to be violent Islamists, although one transnational terrorist group studied in this section that targeted American alongside Japanese targets,

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2112 Restricting totals to U.S. cases sees an increase in the use of firearms.
Spaaij R (2012) *Understanding Lone Wolf Terrorism* Melbourne: Springer.

2113 For a discussion of this study, also see: Paul Gill, *Lone-Actor Terrorists: A Behavioral Analysis*, p. 16-17.

2114 See the aforementioned example of an antitank gun seized in a raid: Jessica Eve Stern, "The Covenant, the Sword, and the Arm of the Lord (1985)," p. 150.

2115 Federal Bureau of Investigation (FBI), "Most Wanted Terrorists: Daniel Andreas San Diego," https://www.fbi.gov/wanted/wanted_terrorists/daniel-andreas-san-diego/view.

2116 Moran H, Costanzo J (1997) "3 animal rights activists are back in court," Deseret News, http://www.deseretnews.com/article/603034/3-animal-rights-activists-are-back-in-court.html?pg=all.

2117 National Consortium for the Study of Terrorism and Responses to Terrorism (START), "Countering Eco-Terrorism in the United States: The Case of 'Operation Backfire'," September 2012, p.12, http://www.start.umd.edu/sites/default/files/files/publications/CountermeasuresOperationBackfire.pdf.

2118 This fact complicates law enforcement infiltration and monitoring of these groups. See: Ibid.

2119 Asal V, Ackerman G, Rethemeyer G (2012), "Connections Can Be Toxic: Terrorist Organizational Factors and the Pursuit of CBRN Weapons," *Studies in Conflict & Terrorism* 35: p. 230.

2120 U.S. Department of State, "Foreign Terrorist Organizations," http://www.state.gov/j/ct/rls/other/des/123085.htm.

2121 The phenomenon that modern terrorism is conducted for radical religious beliefs is what terrorism scholar David C. Rapoport called the fourth or religious wave of terrorism.
Rapoport D (2004) "The Four Waves of Modern Terrorism," in *Attacking Terrorism: Elements of a Grand Strategy*, eds. Audrey Cronin, J. Ludes Washington: Georgetown University Press.

Aum Shinrikyo, was a millenarian cult.[2122,2123] Since transnational terrorist groups have turned toward mass violence and are increasingly displaying a lack of strategic restraint, their members are likely to seek mass casualties and are unlikely to negotiate during attacks.[2124,2125,2126,2127,2128,2129]

The transnational terrorist groups of concern in this section are engaged in very active propaganda and recruitment efforts targeting Western citizens.[2130] Propaganda documents by al Qaeda (central) in particular have called on scientists and technicians to assist them in launching chemical or biological weapons attacks.[2131] Therefore, transnational terrorist groups may seek to recruit US laboratory workers to assist them in attacking a laboratory or may inspire a US laboratory worker to carry out a malicious act in the name of the group.

16.4.5.2 Capabilities

The transnational terrorist groups considered here are well-funded, well-organized, well-armed, and highly motivated groups. They are capable of orchestrating complex attacks and have suitable resources to orchestrate long-term plots. They are able to recruit members willing to carry out suicide operations and mass killings.[2132] They may have a chemical or biological weapons program involving scientifically trained individuals, as was the case with Aum Shinrikyo and al Qaeda (Appendix V: Section 16.7).

The transnational terrorist groups considered as threats in this section have experience with explosives and with a wide range of heavy weapons, and therefore, are capable of breaching secure facilities if such supplies and equipment could be brought to bear in the United States. At the high end of the capability spectrum considered here is the rising Islamic State of Iraq and the Levant (ISIL) group, which controls significant territory in Syria and Iraq and is considered a state-like group (Appendix V: Section 16.9).[2133] This group has demonstrated its ability to recruit or coerce engineers and scientists both in Syria and Iraq and in Western countries (Appendix V: Section 16.9).

2122 Ibid.

2123 In particular, the group attempted to attack two U.S. naval bases in Japan. Richard Danzig, Marc Sageman, Terrance Leighton, Lloyd Hough, Hidemi Yuki, Rui Kotani, Zachary M. Hosford, "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," Center for a New American Security, December 2012, p. 19, http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf.

2124 The degree to which modern groups have become less hierarchical and more prone to mass killings is, however, debated. Hoffman B (2006) *Inside Terrorism* New York: Columbia University Press.

2125 Neumann P (2009) *Old and New Terrorism* Malden: Polity Press.

2126 Duyvesteyn I (2004) "How New is the New Terrorism?" *Studies in Conflict & Terrorism* 27, no. 5: 439-454.

2127 One early work on al Qaeda that remarked how globalized the group had become is:
Peter L. Bergen, *Holy War, Inc.: Inside the Secret World of Osama Bin Laden* (New York: Touchstone, 2001), p. 199-224.

2128 Other case studies restricted to al Qaeda include:
Brad McAllister, "al Qaeda and the Innovative Firm: Demythologizing the Network," *Studies in Conflict & Terrorism* 27 (2004), p. 298-299, 303-306, 314-315;

2129 Jones C (2006) "Al-Qaeda's Innovative Improvisers: Learning in a Diffuse Transnational Network," *Cambridge Review of International Affairs* 19, no. 4.

2130 Hill L, Deveau S, De Vynck G (2014) "Canadians from Calgary to Timmins heed ISIL's tweets," *Bloomberg*, http://www.bloomberg.com/news/articles/2014-10-23/canadians-from-calgary-to-timmins-heed-islamic-state.

2131 Office of the Director of National Intelligence, Bin Laden's Bookshelf," http://www.dni.gov/index.php/resources/bin-laden-bookshelf?start=1;
Retrieved under the "Now Declassified Material" folder: Abu-Salih Al Somali, "Terror Franchise: The Unstoppable Assassin, TECHS Vital role for its success," <http://www.dni.gov/files/documents/ubl/english/Terror%20Franchise.pdf>

2132 Not all groups have the will and capability to carry out suicide attacks. However, suicide attacks have become more common in general, and have been repeatedly employed against U.S. targets in particular. On the diffusion of suicide attacks, see:
Michael C. Horowitz, "Nonstate Actors and the Diffusion of Innovations: The Case of Suicide Terrorism," *International Organization* 64, no. 1 (January 2010): p. 33-64. In particular, note the diffusion diagram, Figure 3, p. 59.

2133 Zachary Laub, Jonathan Masters, "The Islamic State," Council on Foreign Relations Backgrounders, May 18, 2015, http://www.cfr.org/iraq/islamic-state/p14811.

16.4.6 Foreign Intelligence Entities

16.4.6.1 Motivations

Nation-states may want to collect information on US research or actual samples of biological materials through their foreign intelligence arms for a wide range of reasons. Such efforts may be carried out for purely economic gain, as part of economic espionage efforts. They also may be driven by national security matters, such as identifying US biological agent countermeasure capabilities or aggressively attempting to determine whether the the US conducts biological weapons work. In other espionage cases, foreign states may use the information collected to support their own domestic covert biological weapons programs (such as the Soviet Union cases recounted in Appendix V Section 16.2.6).

16.4.6.2 Capabilities

Foreign countries that have targeted the United States in the past have nearly limitless capabilities, including highly-trained scientists, to bear access to research facilities and equipment, pathogenic agents, significant financial resources, and sophisticated cyber-espionage tools. The limiting factor regarding capabilities brought to bear against a US laboratory will be the perceived payoff of the malicious act considered, for instance the perceived value of the information slated to be stolen, and the potential retaliatory consequences if discovered.

16.5 Analysis of Historical Incidents

16.5.1 Assessment of Malicious Act Options for a Lone Outsider

16.5.1.1 Armed Assault

No cases of lone outsiders launching an armed assault against a US laboratory have been uncovered. However, the threat of active shooters on university campuses and other workplaces appears to be increasing, which suggests that this type of attack should not be discounted.

16.5.1.2 Bombing or Arson

Lone outsiders have set off bombs targeting individual biomedical scientists away from research facilities as well as against health care centers, although apparently none against a research laboratory. Ted Kaczynski (the "Unabomber"), acting alone, mailed bombs to several researchers, including to a geneticist (Charles Epstein), in attacks motivated by an anti-modernity and anti-technology ideology.[2134] Eric Robert Rudolph launched a string of bombings in the US against abortion clinics.[2135] Therefore, a bombing or arson attack by an outsider against a laboratory remains a possibility.

The chance of a bombing or arson leading to injury or death can be used as first approximation for the chance of a severe bombing or arson attack that could lead to a loss of containment. This possibility does undercount cases of bombing and arson not intending to cause loss of life either directly through the bombing or indirectly through an outbreak (for instance bombings conducted after-hours, with sole intent

[2134] Fox M (2011) "Charles Epstein, Leading Medical Geneticist Injured by Unabomber, Dies at 77," *The New York Times*, http://www.nytimes.com/2011/02/24/health/research/24epstein.html?_r=0. Accessed July 13, 2015.

[2135] American Association for the Advancement of Science (AAAS), Association of American Universities (AAU), Association of Public and Land-grant Universities (APLU), Federal Bureau of Investigation (FBI), *Bridging Science and Security for Biological Research: Personnel Security Programs*, Meeting Report, Washington, United States, August 21-23, 2013, p. 41-42, http://www.aaas.org/sites/default/files/reports/AAAS-APLU-AAU-FBI%20report%20on%20personnel%20security%20070114.pdf. Accessed July 13, 2015.

to damage the lab); rather, the numbers can be used as a first approximation. Since the Bureau of Labor Statistics began reporting bombing and arson as a separate injury category in 2011, only one such case was reported in the 2011– 2013 timeframe.[2136,2137] The one reported bombing or arson event leading to injury in the 2011– 2013 period did not involve a laboratory (or a hospital).[2138] Since this represents the bombing or arson likelihood across all potential target types in the US, the chance that of a lone outsider carrying out a successful bombing or arson against a laboratory is even lower, in part because high containment laboratories are housed inside of facilities and lack direct access to outside windows or doors. This suggests that a sizable bomb would have to be used to breach containment. Overall, although a bombing or arson by a lone outsider is a technical possibility, the likelihood of its occurrence low, as demonstrated by the lack of known cases and the probabilities cited for context.

16.5.1.3 Covert Entry (Physical) for Theft of Pathogen, Material, Animals, or Information:

No lone outsider case of laboratory theft of pathogen or material has been found in open source reporting. One uncharacterized case that might have been an attempt by an outsider to gain entry covertly involved an attempted theft "targeted at the pathogen collection at the central reference laboratory for animal health in Indonesia" that was "thwarted by security systems installed by the US government," but no further information has been released that would allow one to identify the perpetrator type, motivation, and capability.[2139]

Lone outsiders have surreptitiously obtained pathogens in other ways than theft from a laboratory, as described below. No instances of a lone outsider breaking into a laboratory have been documented in open source reporting, although petty theft (mostly targeting laptops) by outsiders has been anecdotally described.

16.5.1.4 Covert Entry (Cyber) for Theft of Material, Animals, or Information

A covert cyber-entry may be used to steal research information, may facilitate a physical covert entry, or may perhaps even be used to carry out sabotage. For the purposes of this section, the term "material" encompasses digital information.

This section, focuses on the use of cyber-entry to facilitate physical covert entry through access to facility information and security personnel lists and on the potential for cyber-sabotage through access to engineering controls.

Three separate potential targets of cyber-breaches are considered:

1. The first potential target is an individual scientist's personal computer, which is likely to hold research material. Such a breach is statistically likely to occur. Data from 250,000 computers from around the world running on a Windows operating system collected by the security firm Kaspersky showed that approximately 5% of home computers were infected with active,

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[2136] Pre-2011 data on bombing and arson were merged with explosion and fire incidents.
Bureau of Labor Statistics, "Occupational Injuries/Illnesses and Fatal Injuries Profiles: Number of nonfatal occupational injuries and illnesses [...] Bombing, Arson," 2011-2013. Data retrieved at http://data.bls.gov/gqt/InitialPage.

[2137] Bureau of Labor Statistics, "Occupational Injuries/Illnesses and Fatal Injuries Profiles: Fatal occupational injuries [...] Bombing, arson," 2011-2013. Data retrieved at: http://data.bls.gov/gqt/InitialPage. Accessed July 7, 2015.

[2138] This yields a percent chance of a bombing or arson leading to injury or death well below 0.001% per year (alternatively expressed as well below 0.005% over five years and well below 0.015% over 15 years).

[2139] Committee on Prevention of Proliferation of Biological Weapons, Office for Central Europe and Eurasia, National Research Council, *The Biological Threat Reduction Program of the Department of Defense: From Foreign Assistance to Sustainable Partnership* (Washington: The National Academies Press, 2007), p.15, p.15 fn.4.

malicious software (malware).[2140] In 2010, 40% of US households surveyed by a study conducted for Consumer Reports stated that they had had malware on their computer in the last two years.[2141] According to data collected by the market research firm GfK for Consumer Report in 2013, a projected 58.2 million American adults had had at least one malware infection affecting a home computer in 2013.[2142] The overwhelming majority of these infections are not tied to espionage activities, although numerous malicious programs are available even to lone actors that would enable such activities.[2143] The necessary technical skill needed to orchestrate an attack can be relatively low, since certain malware with spying capabilities (spyware) are designed to be user-friendly. For instance, the basic but latest version of the notorious spyware ZeuS was reportedly available for about $700-1000 on the black market, with 24/7 technical assistance offered.[2144,2145]

2. The second potential target is the laboratory's Internet-connected computers, used by researchers to conduct research at the facility. Although these systems may be more protected and monitored than one's home computer, the lab's Internet-connected network will also have more use and hence more risk of infection. A report by the US Office of Management and Budget noted that incidents targeting federal networks of a similar nature had increased from previous years, reaching some 70,000 incidents in 2014.[2146]

3. The third is the laboratory's internal computer network. These computers, in some cases, are "air-gapped," meaning that computers in the internal network and computers in networks that have access to the Internet (and hence might be compromised from the outside) are not connected.[2147] Breaching an air-gapped network would necessitate someone to connect a (likely unknowingly) infected device, such as a USB stick, to a machine on the internal network. Moreover, exfiltrating data out from the air-gapped network would be problematic, and require sophisticated techniques.[2148] For these reasons, malicious actors without physical access to the laboratory are highly unlikely to be able to carry out attacks against air-gapped networks.

If access/security files and device engineering controls are kept on air-gapped networks and if good cyber-security practices are in place regarding access to the air-gapped systems, then the risks posed by lone outsiders can be most likely limited to penetration of the first and second target networks. However, since laboratories exhibited a wide range of device set-ups, laboratories likely vary in their level of cyber security. That said, Biological Select Agents and Toxins laboratories are required to have information

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2140 Kaspersky E (2013) "One in Twenty is the Sad Truth," *Kaspersky Lab* https://eugene.kaspersky.com/2013/03/25/one-in-twenty-is-the-sad-truth/. Accessed July 31, 2015.

2141 "Social insecurity: What millions of online users don't know can't hurt them," *Consumer Reports Magazine*, June 2010, http://www.consumerreports.org/cro/magazine-archive/2010/june/electronics-computers/social-insecurity/overview/index.htm. Accessed July 31, 2015.

2142 Consumer Reports: 58.2 Million Americans Had a Malware Infection on Their Home PC Last Year," *Consumer Reports Magazine*, May 1, 2013, http://pressroom.consumerreports.org/pressroom/2013/05/my-entry.html. Accessed July 31, 2015.

2143 See for instance: "Trojan.Peskyspy- Listening in on your Conversations," *Symantec Official Blog*, August 27, 2009, http://www.symantec.com/connect/blogs/trojanpeskyspy-listening-your-conversations. Accessed July 31, 2015.

2144 Diane Bartz, "Analysis: Top Hacker "retires", experts brace for his return," *Reuters*, October 29, 2010, http://www.reuters.com/article/2010/10/29/us-hackers-zeus-idUSTRE69S54Q20101029. Accessed July 31, 2015.

2145 Macdonald D, ed. Derek Manky, "Zeus: God of DIY Botnets," FortiGuard Center, http://www.fortiguard.com/legacy/analysis/zeusanalysis.html. Accessed July 31, 2015.

2146 Bennett C (2015) "Cyberattacks on federal government hit record high," *The Hill*, http://thehill.com/policy/cybersecurity/234601-cyberattacks-on-government-hit-record-high. Accessed July 31, 2015.

2147 Carrara B, Adams C (2014) "On Acoustic Covert Channels Between Air-Gapped Networks," *Foundations and Practice of Security: 7th International Symposium*, FPS 2014, Montreal, Canada. Revised Selected Papers, eds. Frédéric Cuppens, Joaquin Garcia-Alfaro, Nur Zincir Heywood, Philip W. L. Fong (New York: Springer, 2015), p.4.

2148 Ibid.

security in place to prevent cyber breaches.[2149,2150,2151,2152,2153,2154] Therefore, drawing any conclusions about the potential for sabotage through tampering with engineering controls or grave facilitation of physical access enabled by modification of security personnel lists is difficult.

Penetration of the first two types of target networks, namely personal computers of researchers and Internet-connected laboratory computers, might prove valuable in facilitating unauthorized access to a lone outsider. Access to these systems would reveal sensitive facility information and personal information on facility personnel. Information such as the names and pictures of individuals, project descriptions, personnel schedules, and the exact location of the laboratory within a broader facility could potentially be gleaned from access to these target networks and could facilitate a physical access attempt. Similarly, embarrassing information that might be gleaned from personal computers could be used to subvert an employee through blackmail. However, whether this information could be gathered from open sources is unclear. Interactions with researchers through social media, freely-available aerial imagery of the facility area, descriptions of research projects on lab websites and researcher CVs, and research publications could already provide a strong understanding of the targeted laboratory if combined.

Due to the significant problems surrounding attribution of cybercrimes, no reliable data exists on how many cyber-breaches are the result of a lone malicious actor. In general terms, although there continue to be lone actors who engage in cyber-penetration activities, it appears that hackers are working together more often than in the past.[2155]

16.5.1.5 Sabotage

No instances of a lone outsider breaking into a laboratory have been described in open source reporting (see above discussion under "covert entry"), and, therefore, no lone outsider case of laboratory sabotage was found in open source reporting.

16.5.1.6 Elicitation of Information

Although specific examples of *laboratory* workers being elicited by lone outsiders were not uncovered, the aforementioned example of Eric Robert Rudolph shows that the prospect of a lone outsider eliciting information from an employee to enhance their ability to hit a target cannot be discounted. According to a summary provided by the Federal Bureau of Investigation (FBI), Rudolph "used flattery to befriend young, female temporary employees, new administrative staff, and security guards at [abortion] clinics. Through these techniques, he obtained information regarding security protocols, functions, and scheduling in order to maximize the injurious effects of the attacks on the clinics."[2156]

16.5.1.7 Insertion of Operative

A lone outsider, by definition, does not have access to the laboratory.

2149 42 C.F.R. §73.11(c)(1).
2150 42 C.F.R. §73.11(c)(9).
2151 9 C.F.R. §121.11(c)(1).
2152 9 C.F.R. §121.11(c)(9).
2153 7 C.F.R. §331.11(c)(1).
2154 7 C.F.R. §331.11(c)(9).
2155 Zadig S, Tejay G (2012) "Emerging Cybercrime Trends: Legal, Ethical, and Practical Issues," *Investigating Cyber Law and Cyber Ethics: Issues, Impacts and Practices*, eds. Alfreda Dudley, James Braman, Giovanni Vincenti (Hershey: Information Science Reference.
2156 AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 41-42.

16.5.1.8 Reckless Act…

> *…Infection of outside animals (Wild or domestic):* No cases where a pathogen was taken from a laboratory by an individual insider and used to infect outside animals were found in open sources. However, this incident type cannot be discarded, because, as noted above, lone outsiders have obtained pathogens in the past to commit other crimes.
>
> *…Exposure of lab worker:* This act would require a lone outsider to gain access to a laboratory's pathogen stocks or contained laboratory environment, which, as explained under the covert entry segment above, has not been documented.
>
> *…Infection of public:* The review of confirmed biocrimes from 1990 to 2015 carried out by individuals with no laboratory access highlighted two cases of possession of a dangerous pathogen (Larry C. Ford, Michael Just) and one case of attempted possession (Larry Wayne Harris), alongside several cases of individuals infected with HIV who used their blood to deliberately contaminate others. Overall, lone outsiders have demonstrated the willingness to use pathogens to cause harm if they can obtain them. All but perhaps one case of pathogen possession and attempted possession involved pathogens ordered from culture collections; how Larry C. Ford came to possess dangerous pathogens is unknown. These cases occurred before the advent of stringent dangerous pathogen regulations and hence should not be taken to represent the current capability of a lone outsider to obtain dangerous pathogens.

16.5.1.9 Deliberate Self-Infection:

This act would require a lone outsider to gain access to a laboratory's pathogen stocks or contained laboratory environment, which, as explained under the covert entry segment above, has not been seen in historical case reporting. Furthermore, the desire to commit suicide or harm others through self-infection with a pathogen appears to be extremely low. A single case has been reported in the open literature. The case did not involve a laboratory or a laboratory worker; rather, it involved a woman who attempted suicide through HIV self-infection, probably with the help of an infected friend (see Section 15.2).[2157]

16.5.1.10 Acts for Which No Specific Examples Were Identified in Open Source Reporting:

- Subversion of employee
- Reckless Act involving a point source release of a pathogen from a laboratory
- Reckless Act involving the release of infected laboratory animals from or within the laboratory
- Reckless Act involving infection of laboratory animals outside of containment.
- Reckless Act involving infection of environment

16.5.2 Assessment of Malicious Act Options for a Lone Insider

16.5.2.1 Armed Assault:

The assessment presented under this type of act in the Lone Outsider section holds true with regards to lone insiders. That is, while no cases of lone insiders launching an armed assault against a US were uncovered, such a malicious act could potentially occur in the future. Crimes, including murder, have been committed against individuals at a lab by others from the same lab. The FBI identified one such

[2157] This case is described in: W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 77

recent case: the 2009 murder by asphyxiation of a graduate student named Annie Le by veterinary technician Raymond Clark III.[2158]

16.5.2.2 Bombing or Arson:

No lab bombing or acts of arson caused by a lone insider were found in open source reporting. Based on Bureau of Labor Statistics data cited in the lone outsider section, there was only one reported bombing or arson event leading to injury in the 2011– 2013 period, and it did not involve a laboratory. In overall terms, although a bombing or arson by a lone insider is a technical possibility, the likelihood of its occurrence is low.

16.5.2.3 Covert Entry (Physical):

Disgruntled ex-researcher Mohsen Hosseinkhani provides the historical case underlying this scenario. Although he had already been fired at the time of his crimes, he still held "insider" access to the laboratory, since his access credentials had apparently not yet been revoked.[2159,2160] He leveraged this access to steal equipment and sabotage experiments.[2161]

16.5.2.4 Covert Entry (Cyber):

Please refer to the cyber covert entry in the Lone Outsider overview for a general overview of the potential networks attacked and of the malicious acts that could be facilitated through successfully penetrating these networks.

Unlike lone outsiders, a lone insider is likely to have physical access to computer systems housed in the facility, and may even have physical access to the facility's internal air-gapped network. This greater access facilitates covert entry through the use of malware.

16.5.2.5 Theft of Pathogen:

Four cases of lone insiders stealing pathogens from a laboratory were found in open source reporting. Diane Thompson abused her position as a laboratory technician to steal *Shigella dysenteriae* from the hospital laboratory to infect fellow workers; moreover, she had probably previously stolen a pathogen and used it to infect her boyfriend.[2162] Brian T. Stewart abused his position as a phlebotomist to steal HIV-infected blood from his workplace, which he subsequently injected into his 11-year old son in an attempt to kill him.[2163] Finally, Richard J. Schmidt was a gastroenterologist that injected his former lover with HIV and hepatitis using a contaminated hypodermic syringe obtained from work.[2164]

2158 AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 40.

2159 Anemona Hartocollis, Al Baker, "Doctor Accused of Crimes Against Mice and Lab," *New York Times – City Room Blog*, December 2, 2011, http://cityroom.blogs.nytimes.com/2011/12/02/doctor-accused-of-crimes-against-mice-and-lab/. Accessed July 13, 2015.

2160 "Lab rat switcher jumps bail, flees to Iran," *Iran Times*, http://iran-times.com/lab-rat-switcher-jumps-bail-flees-to-iran/. Accessed July 13, 2015.

2161 Ibid.

2162 Zilinskas R (2011) "Diane Thompson: A Case Study," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas Hoboken: John Wiley & Sons.

2163 Carus W *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*.

2164 AAAS, AAU APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 38.

16.5.2.6 Theft of Material or Information:

Four cases of insiders stealing material from a laboratory were found in open source reporting. The Harvard post-docs, Jiangyo Zhu and Kayako Kimbara, signed a statement admitting that they had stolen research data, cell lines, and genetic material from the laboratory they were working in; the post-docs were transitioning to another laboratory in the United States at the time and wished to use the materials in their research.[2165] Qingqiang Yin attempted to smuggle to China more than 250 vials, test tubes, and petri dishes presumably containing bacteria and yeast that produced a valuable enzyme that he had stolen from a Cornell laboratory he used to work at.[2166] These were placed in his suitcase, and some were leaking, but based on media descriptions of the incident it does not appear that the biological material involved was pathogenic.[2167] Yin presumably did so because he had not been re-hired by the laboratory and he was attempting to obtain a position at a Chinese laboratory.[2168] The case of Mohsen Hosseinkhani described above is an example of an incident where a lone insider stole equipment and non-pathogen biological products (stem cell cultures, antibodies) with commercial and research value.[2169,2170] Hosseinkhani did so for financial gain, but also out of a desire for revenge against having been fired.[2171] Konan Michel Yao stole and attempted to smuggle into the US 22 vials containing DNA encoding Ebola genes taken from his prior employer, the National Microbiology Laboratory (Canada).[2172] He did so in an attempt to transfer his prior research to his new employer.[2173]

16.5.2.7 Sabotage:

Instances of lone insiders sabotaging equipment and experiments have been found in open source reporting. The review of attacks against laboratories and of biocrimes from 1990 to 2015 found two cases of sabotage of equipment and/or experiments committed by lone insiders (Mohsen Hosseinkhani, Vipul Bhrigu) and one case still in trial following a not guilty plea for reason of insanity (Ouyang Xiangyu). Hosseinkham and Bhrigu did not attempt to physically harm anyone; the two incidents were driven instead by a desire for revenge and academic jealousy, respectively. These cases of sabotage did not present a risk of release of a pathogen or of an infected animal. Ouyang Xiangyu was a graduate student at Stanford University who allegedly sabotaged lab mates' research by killing off their stem cells and then proceeded to attempt to poison lab mates and herself by putting paraformaldehyde in their water bottles as well as her own.[2174,2175] She pleaded not guilty due to insanity.[2176]

2165 Holland L (2006) "Couple Admits Cell Line Theft," *The Harvard Crimson*, http://www.thecrimson.com/article/2006/4/17/couple-admits-cell-line-theft-in/. Accessed September 10, 2015.

2166 Choi C (2002) "Lab theft conviction: Former Cornell researcher found guilty of stealing valuable enzymes," *The Scientist*, http://www.the-scientist.com/?articles.view/articleNo/21813/title/Lab-theft-conviction/. Accessed September 10, 2015.

2167 Ibid.

2168 Ibid.

2169 Anemona Hartocollis, Al Baker, "Doctor Accused of Crimes Against Mice and Lab."

2170 "Lab rat switcher jumps bail, flees to Iran," *Iran Times*.

2171 Ibid.

2172 AAAS, AAU APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 37.

2173 Ibid.

2174 Sim M (2015) "A*Star scholarship holder Ouyang Xiangyu expelled from Stanford," *The Straits Times*, http://www.straitstimes.com/singapore/courts-crime/astar-scholarship-holder-ouyang-xiangyu-expelled-from-stanford. Accessed September 10, 2015.

2175 "A*Star scholar charged for poisoning labmates' drinks," April 2, 2015, *TR Emeritus*, https://web.archive.org/web/20150404222631/http://www.tremeritus.com/2015/04/02/astar-scholar-charged-for-poisoning-labmates-drinks/. Accessed September 10, 2015.

2176 Ibid.

16.5.2.8 Elicitation of Information:

Given widespread inter-lab information openness, as promoted by staff safety and security training and by staff presentation of research, a lone insider likely would not need to elicit specific information not already available to them. This situation may be different in select cases where a laboratory is connected to a hospital or where a private industry laboratory conducts compartmentalized commercial work. In such cases, a lone insider may wish to elicit information to allow them to move and transfer pathogens and materials between such compartments (for instance, from the lab to the hospital) to cause some other malicious act (such as stealing research or infecting the public).

16.5.2.9 Subversion of Employee:

The assessment presented under this type of act in the Lone Outsider section holds true with regards to lone insiders. That is, although no cases were identified of lone insiders subverting fellow lab workers, analogous events have occurred before. For instance, a US military police officer arrested in October 2011 for having attempted to sell military secrets had solicited his fellow soldiers for help in the scheme before falling for an FBI sting operation.[2177]

16.5.2.10 Insertion of Operative:

Insiders are, by definition, part of a laboratory.

16.5.2.11 Reckless Act…

> *…Cross-contamination of laboratory animals:* Mohsen Hosseinkhani shuffled the name tags of research animals to sabotage experiments. These animals were not contagious and, hence, there were no risks of cross-contamination. No other relevant cases were identified in open source reporting.
>
> *…Infection of outside animal (Wild or domestic):* No recorded cases were found where an individual insider took a pathogen from a laboratory and used it to infect outside animals. However, this incident type cannot be discarded, because, as noted above, lone insiders have taken pathogens out of laboratories to commit other crimes.
>
> *…Infection of lab worker:* The review of confirmed biocrimes from 1990 to 2015 found one such case (Diane Thompson).
>
> *…Infection of public:* Four confirmed cases involving a pathogen obtained by an individual insider to infect someone from the general public have been committed since 1990. These were the 2001 "Amerithrax" perpetrator(s), Richard J. Schmidt, Brian T. Stewart, and Diane Thompson.[2178]

[2177] Federal Bureau of Investigation (FBI), "Insider Threat- Soldier Receives 16-Year Sentence for Attempted Espionage," April 26, 2013, https://www.fbi.gov/news/stories/2013/april/soldier-receives-16-year-sentence-for-attempted-espionage/soldier-receives-16-year-sentence-for-attempted-espionage. Accessed July 15, 2015.

[2178] FBI strongly believes the perpetrator was a laboratory insider, Bruce Ivins. However, the latter committed suicide before the case could be taken to trial. See: The United States Department of Justice, "Amerithrax Investigative Summary, Released Pursuant to the Freedom of Information Act," February 19, 2010, p. 6-11, 25-92, http://www.justice.gov/archive/amerithrax/docs/amx-investigative-summary.pdf. Accessed July 14, 2015.

16.5.2.12 Deliberate Self-Infection:

No cases of malicious or suicidal self-infection were found in the open literature. This excludes cases of approved scientific self-experimentation. The desire to commit suicide or to harm others through self-infection with a pathogen appears to be extremely low. Only one suicide attempt case has been reported in the open literature, and the case did not involve a laboratory or a laboratory worker. Rather, it was an HIV self-infection case involving a woman who attempted suicide, probably with the help of an infected friend.[2179]

16.5.2.13 Acts for Which No Specific Examples Were Identified in Open Source Reporting:

- Reckless Act involving a point source release of a pathogen from a laboratory
- Reckless Act involving the release of infected laboratory animals from or within the laboratory
- Reckless Act involving infection of laboratory animals outside of containment
- Reckless Act involving infection of environment

16.5.3 Assessment of Malicious Act Options for Organized Criminals

16.5.3.1 Armed Assault:

No cases were uncovered in open source reporting, and an armed assault does not match the perpetrator type since such acts would not generate income and would place the criminals' lives in danger. This actor-act pairing can be discarded as unrealistic.

16.5.3.2 Bombing or Arson:

No cases were uncovered in open source reporting. However, the findings of a 1980 RAND analysis of high-technology or high-value crimes are applicable here, since they describe robberies taking place against secure compounds. The RAND study demonstrated that "perpetrators prefer to threaten violence rather than use it," and that violence was only threatened or used in robberies; in effect, there were no uses of explosives as a means to gain access to a target.[2180]

Attacking a high-containment laboratory would expose the perpetrators to enormous risks. Arson in particular would need to be carried out from the inside of the laboratory to cause significant financial harm (and hence net gain to a competitor), which would require additional risk and sophistication.

Finally, such attacks lack credible monetary gain motivators. A bombing or arson would not generate income, apart from the highly dubious scenario where a rival research group may stand to profit. In sum, bombing and arson attacks by organized criminals against a laboratory are discarded as unrealistic scenarios.

16.5.3.3 Covert Entry (Physical) for Theft of Pathogens or Information:

No cases were uncovered in open source reporting, although organized criminals might potentially carry out such operations in order to steal equipment, pathogens, or information. Organized criminals have attempted to sell weapons-useable, non-biological, material stolen from high-security sites in the past. For instance, criminals have been interdicted in selling a number of vials containing highly-enriched uranium

[2179] This case is described in: W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 77.
[2180] Reinstedt RN, Westbury J "Major Crimes as Analogs to Potential Threats to Nuclear Facilities and Programs".

powder from a facility or facilities in Eastern Europe over the years.[2181] However, theft of dangerous pathogens for resale to terrorists has not been documented in open source documents. Terrorist groups appear to have been unwilling to invest significant funds to support black market demand for pathogens. For example, Al Qaeda's BW program reportedly had a proposed start-up budget of $2000-4000 USD, and one of their principal bioweaponeers routinely complained about a lack of money.[2182,2183] This low profitability and market value for stolen pathogens suggests that organized criminal groups are unlikely to target US laboratories as a source for pathogens for sale on the black market.

Theft of equipment appears unprofitable. Indeed, one case of theft involving what a single individual could carry out of a laboratory amounted to "only" $10,000 of losses.[2184] Although additional individuals may be able to increase their illegal profits by carrying out pieces of heavy equipment present at a laboratory, these items could be stolen from less-protected venues instead.

Overall, laboratories are high-risk, low-reward targets from the perspective of an organized criminal organization.

16.5.3.4 Covert Entry (Cyber) for Theft of Information:

Please refer to the cyber covert entry in the Lone Outsider overview for a general overview of the potential networks attacked and of the malicious acts that could be facilitated through successfully penetrating these networks.

Cyber-espionage by organized crime groups against researchers is uncommon. In their last publicly-available Foreign Economic and Industrial Espionage report dated October 2011, the Office of the National Counterintelligence Executive remarked that: "no evidence of involvement by independent hackers in economic espionage has been found in intelligence or academic reporting to date, in large part due to the absence of a profitable market for the resale of stolen information."[2185] At least one article suggests that a possible organized crime group from Western Europe used computer hacking means to steal information about studies on "biological warfare and nuclear physics" to sell to government entities.[2186] In addition, this group allegedly conducted more typical illegal activities, such as theft of bank account and credit card information.[2187]

16.5.3.5 Sabotage:

The sabotage of a laboratory could conceivably lead to a relative gain for competitors, but the meager profit margins and the high chance of detection make this an unlikely scenario. For instance, although a competitor might derive profits from the elimination of a rival's laboratory, a legal commercial purchase of the rival firm or the hiring of a rival's top scientist would be legal, probably cheaper, more likely to succeed, and far less risky. This actor-act pairing can be discarded as unrealistic.

[2181] The thorough nuclear forensic study of one such vial is described in: Kenton J. Moody, Patrick M. Grant, Ian D. Hutcheon, *Nuclear Forensic Analysis* (Boca Raton: CRC Press, 2005), p. 401-419.

[2182] Ibid.

[2183] Pita R, Gunaratna R (2009) "Revisiting Al-Qa'ida's Anthrax Program," *CTC Sentinel* Vol. 2 Issue 5, https://www.ctc.usma.edu/posts/revisiting-al-qaida%E2%80%99s-anthrax-program. Accessed July 14, 2015.

[2184] AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 42.

[2185] Office of the National Counterintelligence Executive, "Report to Congress on Foreign Economic Collection and Industrial Espionage, 2009-2011," October 2011, p. 10, http://www.ncsc.gov/publications/reports/fecie_all/Foreign_Economic_Collection_2011.pdf. Accessed August 3, 2015.

[2186] Shamah D (2014) "Israeli firm busts 13-year-long Europe hack attack," *Times of Israel*, http://www.timesofisrael.com/israeli-firm-busts-13-year-long-europe-hack-attack/. Accessed August 3, 2015.

[2187] Ibid.

16.5.3.6 Elicitation of Information:

Elicitation of information has been employed by criminal groups before, although no specific cases involving life science laboratories were found in open source literature.[2188] Several sophisticated software suites are available to private citizens that enable aggregating, visualizing, and finding patterns in large amounts of public and non-public information. Criminal hacking groups are believed to be carrying out such "dossier-building" activities to facilitate future hacks.[2189] Information obtained through elicitation, in particular through orchestrated social media interactions, can potentially be incorporated into these dossiers.

16.5.3.7 Subversion of Employee:

No cases involving a criminal group subverting an employee at a life science laboratory were uncovered in open source reporting. The aforementioned 1980 RAND analysis of high-technology or high-value crimes demonstrated that the number of insiders that participate in a theft increases with the expected illegal profit.[2190] Coercion of employees by criminal groups has occurred before when the payoff was believed to be very high; for instance, robbers have targeted the families of bank managers in an attempt to coerce the latter to assist in particular robberies.[2191] These gambits are complex, expensive, and personnel-intensive operations for criminal groups to carry out, as at least two teams (the hostage takers and the robbers) must work in coordination and must have conducted extensive reconnaissance to carry out such an attempt. Since as noted above, laboratory thefts are likely not profitable, organized criminals would have difficulty subverting insiders, and are unlikely to coerce employers.

16.5.3.8 Reckless Act…

> *…Infection of outside animal (Wild or domestic):* This scenario has occurred previously. In one historical case, a pathogen was illicitly obtained by two criminals (Kevin T. Birch and James B. Cahoon), and it was hypothesized that their end goal was to kill a race horse.[2192] In another case, New Zealand farmers admitted to having illegally introduced rabbit haemorrhagic disease for use as a bio-control tool, after the use of the pathogen as a bio-control tool had been rejected by the New Zealand Ministry of Agriculture and Forestry.[2193] Although this last case stretches the definition of "organized crime," it is still a crime committed by a group of individuals for financial gain, albeit an indirect one (by having more crops to sell, since less crops would be lost to rabbits).
>
> One potential additional case exists, but details remain insufficient to rule either way. Russian officials told visiting US National Research Council committee members in March 2007 that the Russian Prosecutor's office in Moscow had launched an investigation that year into "alleged unsuccessful efforts to attack a large suburban chicken marketplace by introducing chicken affected by avian influenza virus, which would cause the marketplace to close and business to

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2188 Federal Bureau of Investigation (FBI), "Internet Social Networking Risks," https://www.fbi.gov/about-us/investigate/counterintelligence/internet-social-networking-risks. Accessed August 11, 2015.
2189 Robert Graham, "Because dossiers," *Errata Security*, June 16, 2015, http://blog.erratasec.com/2015/06/because-dossiers.html#.VbfWTPnZViY. Accessed August 11, 2015.
2190 Reinstedt RN, Westbury J (1980) "Major Crimes as Analogs to Potential Threats to Nuclear Facilities and Programs"..
2191 Ibid.
2192 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, Working Paper (Washington Center for Counterproliferation Research, National Defense University, February 2001 Revision), p. 101.
2193 Ibid.

shift to a competing marketplace."[2194] Russian media reports on this event presented a different perspective, specifically that the investigation was for "violation of veterinary rules which negligently caused the spread of epizootic diseases or other serious consequences." However, this statement contradicts previous statements made by a prosecutor who told the Russian media that he was not ruling out the possibility that the infected birds sold at the market had been "infected intentionally shortly before the sale."[2195] The results and scope of this investigation are not known.

…*Exposure of lab worker:* No cases were uncovered in open source reporting. No realistic scenarios exist where such acts would both generate significant illegal profits and do so in a manner that could not be conducted in an easier manner. This actor-act pairing can be discarded as unrealistic.

…*Infection of public:* No cases have been uncovered in the open source literature on the use, attempted use, acquisition, attempted acquisition, or development of pathogens as weapons *against individuals* by organized crime groups.[2196] The use of chemical poisons, including toxins, by organized crime groups has been documented. For instance, Chinese and Russian contract killers have reportedly used a toxin derived from the Gelsemium plant genus to poison their victims.[2197] Although information is scant, poisons are probably used by organized crime groups because: they are comparatively easy to conceal and use against a target without endangering the assassin; they are very likely to kill once introduced into the victim's system; the delayed onset of symptoms of some poisons provides time for the perpetrator to escape; and because the use of a rare poison has a chance to be missed in an autopsy. These characteristics are not completely shared with the pathogens investigated in GoF laboratories.

16.5.3.9 Deliberate Self-Infection:

No cases were uncovered in open source reporting. Such acts do not match the perpetrator type, as no realistic scenarios exist where such acts would generate significant illegal profits and do so in a manner that could not be conducted in an easier manner.

16.5.3.10 Acts for Which No Specific Examples Were Identified in Open Source Reporting:

- Insertion of an operative (the significant time and resources needed would go beyond most organized crime groups' resources),
- Reckless Act involving a point source release of a pathogen from a laboratory (these acts do not match the perpetrator type),
- Reckless Act involving the release of infected laboratory animals from or within the laboratory (these acts do not match the perpetrator type),

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[2193] Committee on Prevention of Proliferation of Biological Weapons, Office for Central Europe and Eurasia, National Research Council, *The Biological Threat Reduction Program of the Department of Defense: From Foreign Assistance to Sustainable Partnership* (Washington: The National Academies Press, 2007), p.15, p.15 fn.4.

[2195] Михаил Алексеев [Mikhail Alekseyev], "Пернатая зараза [Fowl Infection]," *Lenta.ru*, February 19, 2007, http://lenta.ru/articles/2007/02/19/flu1/. Accessed October 15, 2015.

[2196] For instance, the following review article on organized crime's multi-faceted challenges to public health did not raise such scenarios: Lucy Reynolds, Martin McKee, "organised crime and the efforts to combat it: a concern for public health," *Global Health* 6 (2010): p. 21, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2996357/. Accessed July 13, 2015.

[2197] Whitehead T (2015) "Fears Russian tycoon Alexander Perepilichnyy may have been poisoned with rare plant," *Telegraph*, http://www.telegraph.co.uk/news/uknews/11614054/Fears-Russian-tycoon-Alexander-Perepilichnyy-may-have-poisoned-with-rare-plant.html. Accessed July 13, 2015.

- Reckless Act involving cross-contamination of laboratory animals (these acts do not match the perpetrator type), and
- Reckless Act involving infection of laboratory animals outside of containment (these acts do not match the perpetrator type).

16.5.4 Assessment of Malicious Act Options for Domestic Terrorists and Extremists

16.5.4.1 Armed Assault:

No such cases were found in open source reporting. A review of cases of attacks on laboratories (see Section 15.1) shows that laboratories have been frequent targets of animal rights extremists, principally by the Animal Liberation Front (ALF) and sometimes by the Earth Liberation Front (ELF). As noted above, both ALF and ELF doctrinal documents reject the killing of individuals.[2198] ALF has never used firearms in attacks. However, other eco-radical groups, such as the defunct US-based R.I.S.E. cell (see Section 15.5) and the Mexican-based eco-anarchist group Individuals Tending to Savagery, have been more inclined towards violence.[2199,2200] Therefore, although an armed assault against a laboratory by a domestic terrorist or extremist group would be a novel occurrence, it remains a viable scenario.

16.5.4.2 Bombing or Arson:

Arson attacks are a trademark of the ALF, as well as of eco-radical groups like the ELF.[2201] The review of recent attacks (from 1989– 2015) against laboratories (see Section 15.1) documents five arson attacks against US laboratories.

Although no bombings at US biological laboratories were uncovered in open source reporting, commercial buildings owned by biotechnology companies have been bombed in the United States. Daniel Andreas San Diego is on the FBI's Most Wanted list for having allegedly planted bombs against two biotechnology companies that had commercial ties with Huntington Life Sciences; he remains on the run.[2202] FBI believes San Diego to be "involved with" the Stop Huntington Animal Cruelty group, hence the inclusion of this case under the domestic terrorists and extremists section.[2203] More specifically, he is wanted for the bombing of the Chiron Life Science Center and the Shaklee Corporation building, both in California in 2003.[2204,2205,2206] The bomb used against Shaklee Corporation was designed to produce shrapnel through the addition of nails around the explosive charge, while the attack against Chiron included a secondary bomb in a possible attempt at targeting first responders.[2207]

2198 Ackerman G (2003) "Beyond Arson? A Threat Assessment of the Earth Liberation Front," *Terrorism and Political Violence* Vol. 15, 4.
2199 Phillips L (2012) "Anarchists attack science," *Nature (News)* 485, no. 561, http://www.nature.com/news/anarchists-attack-science-1.10729 Accessed September 11, 2015.
2200 Corral G (2011) "Stand up against the anti-technology terrorists," *Nature (News)* 476, no. 373, http://www.nature.com/news/2011/110822/full/476373a.html. Accessed September 11, 2015.
2201 Ibid.
2202 Federal Bureau of Investigation (FBI), "New Most Wanted Terrorist: First Domestic Fugitive Added to List," April 21, 2009, https://www.fbi.gov/news/stories/2009/april/wanted_042109. Accessed August 27, 2015.
2203 Ibid.
2204 Ibid.
2205 Federal Bureau of Investigation (FBI), "Terrorism 2002-2005," p.9-10.
2206 Rodriguez M, Chong JR, Krikorian G (2003) "Suspect is Sought in Bombings," *Los Angeles Times*, http://articles.latimes.com/2003/oct/10/local/me-warrant10. Accessed 28, 2015.
2207 Federal Bureau of Investigation (FBI), "New Most Wanted Terrorist: First Domestic Fugitive Added to List."

In addition, eco-radical groups in Latin America and Europe have targeted nanotechnology researchers by sending mail bombs to the researchers' laboratories.[2208,2209] These groups have issued propaganda against synthetic biology research, and as such could conceivably target biological researchers in the future.[2210] So far, these groups have not targeted US laboratories or US researchers.

16.5.4.3 Covert Entry (Physical):

ALF has repeatedly covertly entered laboratories (see Section 16.1), although none of the facilities breached were secured at the current high containment or Biological Select Agents and Toxins levels.

16.5.4.4 Covert Entry (Cyber):

Please refer to the cyber covert entry in the Lone Outsider overview for a general overview of the potential networks attacked and of the malicious acts that could be facilitated through successfully penetrating these networks.

Cyber-operations by terrorists have so far been largely limited to simplistic attacks, such as website defacement.[2211] Director of National Intelligence James R. Clapper remarked in the latest 2015 unclassified Worldwide Threat Assessment of the US Intelligence Committee that: "terrorist groups will continue to experiment with hacking, which could serve as the foundation for developing more advanced capabilities. Terrorist sympathizers will probably conduct low-level cyber-attacks on behalf of terrorist groups and attract attention of the media, which might exaggerate the capabilities and threat posed by these actors."[2212] Based on these remarks, domestic extremist or terrorist groups are currently judged incapable of sabotaging laboratories through the hijacking of engineering control systems, which would require a sophisticated cyber-attack. In addition to the complexity of the required attack code needed to interact with the engineering control systems, such an attack could require the penetration of an air-gapped network if the engineering controls are on an isolated intranet.

16.5.4.5 Theft of Pathogen:

Although ALF has covertly entered laboratories, no open source reports documented a case of theft of pathogen (see Section 16.3).

16.5.4.6 Theft of Material, Animals, or Information:

ALF has stolen research documents from laboratories and individuals in a direct attempt to disrupt research they oppose.[2213] ALF has stolen animals from facilities, but they have not stolen from high

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[2208] Phillips L (2012) "Anarchists attack science," *Nature (News)* 485, no. 561, http://www.nature.com/news/anarchists-attack-science-1.10729 Accessed September 11, 2015.

[2209] Corral G (2011) "Stand up against the anti-technology terrorists," *Nature (News)* 476, no. 373, http://www.nature.com/news/2011/110822/full/476373a.html. Accessed September 11, 2015.

[2210] Ibid.

[2211] Theohary C, Rollins J (2011) "Terrorist Use of the Internet: Information Operations in Cyberspace," *Congressional Research Service*, https://www.fas.org/sgp/crs/terror/R41674.pdf. Accessed August 3, 2015.

[2212] Director of National Intelligence, James R. Clapper, Statement for the Record- Worldwide Threat Assessment of the U.S. Intelligence Community, Senate Armed Services Committee, February 26, 2015, p. 3, http://www.dni.gov/files/documents/Unclassified_2015_ATA_SFR_-_SASC_FINAL.pdf. Accessed August 3, 2015.

[2213] "Lab Records, Dogs Stolen From Baby Fae Surgeon," *Los Angeles Times*, August 16, 1988, retrieved at *Orlando Sentinel*, http://articles.orlandosentinel.com/1988-08-16/news/0060190259_1_baby-fae-linda-university-loma-linda. Accessed August 3, 2015.

contentment facilities (see Section 16.1). They have also stolen animal cages to aid in the exfiltration of animals from facilities (see Section 16.1).[2214]

16.5.4.7 Sabotage

ALF and other animal rights extremist groups have typically sabotaged buildings they have broken into. Five cases of laboratory sabotage (excluding arson) are documented in all five were carried out by ALF. These acts were not covert and were not intended to injure. However, since these groups often destroyed machinery to prevent continued experiments, the risk of an accidental release from sabotage cannot be ruled out.

16.5.4.8 Elicitation of Information:

Elicitation is likely to have been carried out in planning domestic terrorist and extremist attacks, although this type of information is rarely documented in open sources. FBI has noted that "ALF activists will not merely attack a university where animal research is conducted, but rather will attempt to locate the specific laboratory at the university where the research is being conducted [...]."[2215] Elicitation is one potential method that can be used find out which laboratory is conducting animal research, but is not the sole means of doing so. A memoir by an ALF member makes mention of students providing information to ALF "moles" during the planning phase of a laboratory attack; the use of the term "mole" suggests that the students were being elicited by ALF.[2216] As such, domestic extremist groups appears to have used elicitation on more than one occasion.

16.5.4.9 Subversion of Employee:

ALF is believed to have either subverted employees or inserted operatives on several occasions. FBI has stated that animal rights extremists have "obtain[ed] proprietary or confidential information about intended victim companies through theft or from sympathetic insiders."[2217] Camera footage from one laboratory break-in perpetrated by the group showed members with access keys, while another break-in without signs of forced entry left police wondering if the group had a physical key.[2218,2219] A memoir by an ALF member mentions "moles" providing information to the group.[2220] An account by a supporter of

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[2214] Statement of Senator David Vitter, "opening statement," Oversight on Eco-terrorism specifically examining the Earth Liberation Front ("ELF") and the Animal Liberation Front ("ALF"). U.S. Senate Committee on Environment & Public Works, May 18, 2005, http://www.epw.senate.gov/pressitem.cfm?party=rep&id=237834, http://www.epw.senate.gov/hearing_statements.cfm?id=237836. Accessed August 11, 2015.

[2215] Federal Bureau of Investigation (FBI), "Terrorism 2000/2001," p. 27.

[2216] Anonymous, *Memories of Freedom: Western Wildlife Unit of the Animal Liberation Front*, p. 21, http://theanarchistlibrary.org/library/western-wildlife-unit-of-the-animal-liberation-front-memories-of-freedom.pdf. Accessed October 15, 2015.

[2217] John E. Lewis, Deputy Assistant Director, Federal Bureau of Investigation (FBI), Testimony Before the Senate Judiciary Committee, Washington, U.S.A, May 18, 2004, https://www.fbi.gov/news/testimony/animal-rights-extremism-and-ecoterrorism. Accessed August 11, 2015.

[2218] McGlynn A (2009) "Activist who refused grand jury testimony now charged with conspiracy," *Lancaster Online*, http://lancasteronline.com/your_news/community/activist-who-refused-grand-jury-testimony-now-charged-with-conspiracy/article_3e187816-29c4-5c46-89aa-6edfbf8c8cfc.html?mode=jqm. July 13, 2015.

[2219] Sorensen E "Activists vandalize WSU labs, release research animals," *The Spokesman-Review*, A1, A7, retrieved at: Animal Liberation Frontline, Animal Liberation Front Press Clippings 1984-1994, p.20-21, http://animalliberationfrontline.com/wp-content/uploads/2014/10/ALF-News-Article-Collection.pdf. Accessed October 15, 2015.

[2220] For example: "ALF moles followed up on leads of other potential targets, and searched veterinary medicine files for possible future actions." In: Anonymous, *Memories of Freedom: Western Wildlife Unit of the Animal Liberation Front*, p.15, 17, 19, 21, http://theanarchistlibrary.org/library/western-wildlife-unit-of-the-animal-liberation-front-memories-of-freedom.pdf. Accessed October 15, 2015.

the group talks of "the A.L.F.'s source inside the lab" when discussing another group action.[2221] In the UK, animal rights extremists have subverted a civil servant at the Driver and Vehicle Licensing Agency to obtain addresses of the individuals they were targeting.[2222] The individual apparently provided such information out of sympathy for animal rights protests; his legal defense argued that he had "believed the information would be used for lawful protest."[2223] As such, domestic extremist groups appear to have subverted employees on several occasions.

16.5.4.10 Insertion of Operative

In addition to the probable ALF cases noted under the "subversion of employee" entry above, R.I.S.E.'s co-founder Stephen J. Pera probably joined a research group to gain access to pathogen-growing equipment (see Section 16.5).

16.5.4.11 Reckless Act…

…*Pathogen point-source release from lab:* No such cases were found in open source reporting, although the potential for an event of this type cannot be excluded.

…*Release of infected laboratory animals from the laboratory:* ALF has released lab animals on numerous occasions, and has also exfiltrated animals themselves out of the lab, as "animal liberation" is one of the group's top priority (see Section 15.1). In a 1987 case, animal rights extremists calling themselves the Band of Mercy stole eleven cats infected with *Toxoplasma gondii* and other uninfected animals from a research laboratory.[2224] The members reportedly knew that the cats were infected at the time of the theft, but the group reportedly gave assurances that the cats had been put under veterinary care after the break-in.[2225] In a subsequent 1989 case, ALF stole mice that were infected with cryptosporidium from a research laboratory.[2226] The perpetrators claimed in a press release that "absolutely no animals were released into the community," that "all animals were carefully transported to safe houses," and that "the infected mice were […] being treated."[2227] Based on these historical examples, the possibility that infected animals could be released from the laboratory cannot be ruled out. The potential consequences of such a release would be contact of infected animal with people, other lab animals, and/or wild animals that could lead to an outbreak.

…*Cross-contamination of laboratory animals:* This event has apparently not occurred previously, although ALF has mixed animal cages before in an attempt to disrupt experiments (see Section 15.1). Should some of the animals be infected with a contagious disease, the practice could cross-

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2221 "Blast from the Past- '80s Lab Raids," *No Compromise* 15, http://www.nocompromise.org/issues/15blast_past.html. Accessed October 15, 2015.

2222 Fenton B (2004) "DVLA mole jailed for aiding guinea pig farm activists," *The Telegraph*, http://www.telegraph.co.uk/news/uknews/1475082/DVLA-mole-jailed-for-aiding-guinea-pig-farm-activists.html. Accessed October 15, 2015.

2223 Ibid.

2224 Schneider K (1987) "Theft of Infected Cats From U.S. Lab Spurs Alert," *The New York Times*, http://www.nytimes.com/1987/08/25/us/theft-of-infected-cats-from-us-lab-spurs-alert.html. Accessed October 15, 2015.

2225 As given in an account "intended to represent the views of the United States Animal Liberation Front and its members." Ingrid Newkirk, *Free the Animals: The Amazing True Story of the Animal Liberation Front* (New York: Lantern Books, 2000). p. 339-355, front matter, and pictures.

2226 "Diseased mice freed in arson fires, break-in," *Spartanburg Herald-Journal*, April 4, 1989, A2. Retrieved at: https://news.google.com/newspapers?nid=1876&dat=19890404&id=2kwsAAAAIBAJ&sjid=Vs4EAAAAIBAJ&pg=6664,1859692&hl=en. Accessed June 26, 2015.

2227 Animal Liberation Frontline, Animal Liberation Front Press Clippings 1984-1994, p. 29, http://animalliberationfrontline.com/wp-content/uploads/2014/10/ALF-News-Article-Collection.pdf. Accessed October 15, 2015.

contaminate laboratory animals. Whether ALF would deliberately cross-contaminate facilities, given that the outcome would likely cause harm to some uninfected animals and, hence, violate ALF guidelines is unclear. However, some ALF members have in practice treated animals within the raided facilities as already dead, justifying acts that impede the operation of the facility despite the fact that these actions also pose disproportionate risk to the held animals. This attitude is most visible in ALF attacks against mink farms, where the animals released have very little chance to survive in the wild and often end up dead on roads.[2228,2229,2230,2231]

…Infection of outside animal (Wild or domestic): No such cases were found in open source reporting, although the 30 infected mice released in the aforementioned 1989 ALF raid had the potential to spread cryptosporidium for a week to ten days, both through direct contact and through mice feces.[2232]

…Infection of lab worker: No such cases were found in open source reporting. A website dedicated to the ALF alleges that "vials of infectious serum were removed from a refrigerator [and left] to spoil" in one alleged 1998 break-in at a private research laboratory.[2233] No open source information is available on this alleged case, and no open source documentation is available to confirm that an attack against the laboratory took place. Indeed, the incident is not included in FBI's public list of domestic terrorist and extremist incidents, unlike other ALF attacks.[2234] The apparent support of such a tactic in pro-ALF circles nevertheless raises the possibility that pathogen vials could be opened and spread out in a laboratory during an ALF attack, an event which could lead to the infection of a lab worker or a first responder.

…Infection of public: Only two domestic terrorist or extremist groups (Rajneeshee Cult, R.I.S.E.) have sought a biological weapons capability. Both the Rajneesh Cult and R.I.S.E. are long-defunct groups whose history is documented in Section. 15.5. Section. 15.3, and Section 15.6 provide data on these groups. Overall, these groups were able to begin a BW program because they could leverage their access to lab pathogens. Their programs were extremely rudimentary from a technical standpoint, although the Rajneesh group's efforts were highly effective. In addition, one (also long-defunct) right-wing supremacist group placed medical waste near a Jewish organization as part of a hate crime that threatened infection (see Section 15.6).

The number of domestic terrorist or domestic extremist groups or members that are active in the US is not available in open source reporting, since neither the FBI nor the Department of Justice release such statistics. However, The New America Foundation maintains a running tally of "homegrown extremism" incidents since 2001, and as of 2015 they had identified 479

2228 "Thousands of mink freed in B.C. in apparent act of 'eco-terrorism'," *Vancouver Province*, August 27, 2008, http://www.canada.com/reginaleaderpost/news/story.html?id=7d4845f1-4bc7-4162-bb57-4919aff76869. Accessed August 3, 2015.

2229 Carbery G (2010) "Investigation under way after 5,000 mink freed from farm," *The Irish Times*, http://www.irishtimes.com/news/investigation-under-way-after-5-000-mink-freed-from-farm-1.656730. Accessed August 3, 2015.

2230 Minkfarm drabbad av utsläppta djur." *Småland*, October 10, 2010, http://sverigesradio.se/sida/artikel.aspx?programid=105&artikel=4086537. Accessed August 3, 2015.

2231 "Nuovo blitz degli animalisti Liberati 1.400 visoni da pelliccia." *Gazzetta di Mantova*, January 18, 2012, http://gazzettadimantova.gelocal.it/mantova/cronaca/2012/01/18/news/nuvo-blitz-degli-animalisti-liberati-1-400-visoni-da-pelliccia-1.3080658. Accessed August 3, 2015.

2232 "Diseased mice freed in arson fires, break-in," *Spartanburg Herald-Journal*.

2233 "Laboratory Animal Liberation Campaign," *Animal Liberation Front*, http://www.animalliberationfront.com/ALFront/lab.htm. Accessed August 11, 2015.

2234 For ALF events cited in FBI's compendiums, see for example the "FBI's Terrorism in the United States 1999" list. FBI, "Terrorism in the United States 1998," p.1-24, https://www.fbi.gov/stats-services/publications/terror_98.pdf. Accessed August 28, 2015.

"homegrown extremists" involved in 35 carried-out plots of which 26 were lethal incidents, and 131 interdicted plots.[2235] Comparing this large number to the few cases of BW-related terrorist and extremist incidents logged in Section 15.3, demonstrates that domestic terrorist or extremist groups rarely seek the capability to infect the public, let alone to carry out acts that have the potential to cause infection with laboratory-derived pathogens.

16.5.4.12 Deliberate Self-Infection:

No such cases were uncovered in open source reporting. The motive behind a domestic terrorist or extremist group member deliberately self-infecting would most likely be limited to infecting others (i.e., not suicide or unsanctioned experimentation). As discussed in the "infection of public" entry above, only two domestic terrorist or extremist groups have sought to infect others and neither considered self-infection as a means of doing so.

16.5.5 Assessment of Malicious Act Options for Transnational Terrorists, including State-Like Groups

16.5.5.1 Armed Assault

No cases reported involved a US-operated or US-owned lab. One case of armed assault conducted by transnational terrorists against a non-US lab, a heavily-defended defense-related installation in Yemen, was found in open source reporting (see Section 15.1). In that case, a military hospital was attacked by members of Al Qaeda in the Arabian Peninsula as part of a broader breach of a military compound, and the group killed doctors and patients inside.[2236] The group later apologized for having done so and claimed that a fighter had disobeyed orders in targeting the hospital rather than focusing on the military targets at the compound.[2237] Terrorist groups have often launched armed assaults against hospitals overseas, which often have a diagnostic laboratory.[2238] However, this act was typically executed to cause maximum casualties and/or to take many hostages at once, and in no identified cases were pathogens smuggled out.[2239]

16.5.5.2 Bombing or Arson

No cases reported involved a US-operated or US-owned lab. Transnational terrorists have carried out several bombing attacks against non-US labs, including the aforementioned attack against one heavily-defended defense-related installation in Yemen (which was a combined suicide car bomb – armed assault attack). Section 15.1 contains a summary of three such cases. Numerous additional cases of bombings targeting hospitals have been described in open sources.[2240]

[2235] The New America Foundation International Security Program, "Homegrown Extremism 2001-2015," http://securitydata.newamerica.net/extremists/analysis.html, http://securitydata.newamerica.net/extremists/deadly-attacks.html, http://securitydata.newamerica.net/extremists/terror-plots.html, http://securitydata.newamerica.net/extremists/methodology.html. Accessed June 30, 2015.

[2236] Nasser Arrabyee, Ben Hubbard, "Attack on Yemen's Defense Headquarters Is Linked to Al Qaeda," *The New York Times*, December 6, 2013, http://www.nytimes.com/2013/12/07/world/middleeast/yemen-attack.html. Accessed August 21, 2015

[2237] The group claimed to be targeting alleged drone control rooms and American experts at the site. Associated Press, "Al Qaeda Branch in Yemen Regrets Hospital Attack," *Associated Press* through *The New York Times*, December 22, 2013, http://www.nytimes.com/2013/12/23/world/middleeast/al-qaeda-branch-in-yemen-apologizes-for-attack-on-hospital-at-defense-ministry.html. Accessed August 21, 2015.

[2238] Boaz Ganor, Miri Halperin Wernli, "Terrorist Attacks against Hospitals Case Studies," *International Institute for Counter-Terrorism*, October 27, 2013, p. 1-32, http://www.ict.org.il/Article/77/Terrorist-Attacks-against-Hospitals-Case-Studies. July 13, 2015.

[2239] Ibid.

[2240] Ibid.

16.5.5.3 Covert Entry (Physical) for Theft of Pathogens or Information:

No such cases were found in open source reporting, although numerous transnational terrorist groups have carried out covert infiltrations to hit their targets. The lack of cases of covert entry is, therefore, simply a byproduct of the relative lack of attacks against laboratories. According to a National Research Council publication, unspecified terrorist websites have "suggested that their operatives can pose as students to gain access to university laboratories and remove hazardous chemical, biological, or radiological agents."[2241] Although this statement suggests that at least some low-level interest among transnational terrorists in covert entries at university laboratories exists, whether US laboratories are considered for attack is unclear.

16.5.5.4 Covert Entry (Cyber)

Refer to the cyber covert entry in the Lone Outsider overview for a general overview of the potential networks attacked and of the malicious acts that could be facilitated through successfully penetrating these networks.

Cyber-operations by terrorists have so far been largely limited to simplistic attacks, such as website defacement.[2242] Director of National Intelligence James R. Clapper remarked in the latest 2015 unclassified Worldwide Threat Assessment of the US Intelligence Committee that: "terrorist groups will continue to experiment with hacking, which could serve as the foundation for developing more advanced capabilities. Terrorist sympathizers will probably conduct low-level cyber-attacks on behalf of terrorist groups and attract attention of the media, which might exaggerate the capabilities and threat posed by these actors."[2243] Based on these remarks, transnational terrorist groups are currently judged incapable of sabotaging laboratories through the hijacking of engineering control systems, which would require a sophisticated cyber-attack. In addition to the complexity of the required attack code needed to interact with the engineering control systems, such an attack could require the penetration of an air-gapped network if the engineering controls are on an isolated intranet.

16.5.5.5 Sabotage

No known instances of sabotage of a laboratory by transnational terrorists were found in open source reports (see Section 15.3). Should a group launch an armed assault against a laboratory, they are likely to carry out overt sabotage once within a laboratory, which may in turn lead to a breach in containment. For instance, when transnational groups capture a high-profile location and/or hold large numbers of hostages, they often set explosives to complicate hostage rescue.[2244,2245,2246] A group that captures a laboratory as part of a negotiating strategy should be expected to set explosives. The charges could be detonated, either by the terrorists, or accidentally as part of a rescue attempt gone wrong. This event has occurred before,

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2241 National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version* (Washington: The National Academies Press, 2011), p. 260.

2242 Theohary C, Rollins J (2001) "Terrorist Use of the Internet: Information Operations in Cyberspace," *Congressional Research Service*, https://www.fas.org/sgp/crs/terror/R41674.pdf. Accessed August 3, 2015.

2243 Director of National Intelligence, James R. Clapper, Statement for the Record- Worldwide Threat Assessment of the U.S. Intelligence Community, Senate Armed Services Committee, February 26, 2015, p. 3, http://www.dni.gov/files/documents/Unclassified_2015_ATA_SFR_-_SASC_FINAL.pdf. Accessed August 3, 2015.

2244 A few recent, high profile, examples include: Lamine Chikhi, Bate Felix, "Sahara Islamists take hostages, spreading Mali war," *Reuters*, January 16, 2013, http://www.reuters.com/article/2013/01/16/us-sahara-crisis-idUSBRE90F1JJ20130116. Accessed July 15, 2015.

2245 "When kids bury kids': Russia remembers 130 victims of Nord-Ost terror act 10 years on," *Russia Today*, October 23, 2012, http://rt.com/news/nord-ost-terror-anniversary-827/. Accessed July 15, 2015.

2246 "'I don't feel guilty': Single surviving Beslan terrorist unrepentant 10 years after tragedy," *Russia Today*, September 1, 2014, http://rt.com/news/184044-only-surviving-beslan-terrorist/. Accessed July 15, 2015.

for example when the terrorists detonated charges during the Beslan hostage crisis.[2247] An internal explosion could breach containment walls, damage filtration and air pressure systems, breach animal pens, and breach infected waste storage and disposal systems. In turn, hostages, responders, the general public, and the terrorists themselves could be exposed to pathogens.

16.5.5.6 Elicitation of Information

Elicitation is likely to have been carried out in planning some transnational terrorist attacks, although this type of information is not documented in open sources.

16.5.5.7 Subversion of Employee

Same as for "Covert entry," *mutatis mutandis*.

16.5.5.8 Insertion of Operative

Same as for "Covert entry," *mutatis mutandis*.

16.5.5.9 Reckless Act…

> *…Pathogen point-source release from lab:* No such cases were found in open source reporting However, transnational groups have often planted explosives in captured buildings, including hospitals, in an effort to complicate hostage rescue.[2248] As noted above, a scenario where a group captures a laboratory to use as a negotiating strategy may degenerate into a pathogen point-source release even if this was not the end goal of the terrorists. This outcome could be deliberately occasioned by the terrorists themselves, or the result of a law enforcement response gone awry.
>
> *…Release of infected lab animals from within the lab:* No such cases were found in open source reporting.
>
> *…Cross-contamination of laboratory animals:* No such cases were found in open source reporting.
>
> *…Infection of lab animals outside of containment:* No such cases were found in open source reporting.
>
> *…Infection of outside animal (Wild or domestic):* No such cases were found in open source reporting, although Al Qaeda apparently considered some type of attack against US agriculture given that "hundreds of pages of US agricultural documents" were apparently recovered from Al Qaeda hideouts in Afghanistan.[2249,2250,2251]

[2247] Ekaterina Stepanova, "From Dubrovka to Beslan: Who is learning faster?," PONARS Policy Memo 347, November 2004, p.3, http://csis.org/files/media/csis/pubs/pm_0347.pdf. Accessed July 15, 2015.

[2248] Ibid.

[2249] Reported by: Susan Collins, "Opening Statement," in *Agroterrorism: The Threat to America's Breadbasket*, Senate Committee on Governmental Affairs, S.Hrg. 108-491, Nov. 19, 2003, http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=108_senate_hearings&docid=f:91045.wais.pdf. Accessed July 14, 2015.

[2250] Jim Monke, "Agroterrorism: Threats and Preparedness," CRS Report for Congress, March 12, 2007, p.1-56, https://www.fas.org/sgp/crs/terror/RL32521.pdf. Accessed July 14, 2015.

[2251] R. Goodrich Schneider et al., "Agroterrorism in the U.S.: An Overview," University of Florida Food Science and Human Nutrition Department, Florida Cooperative Extension Service, FSHN0521, August 2009, https://edis.ifas.ufl.edu/fs126. Accessed July 14, 2015.

...Infection of lab worker: No such cases were found in open source reporting.

...Infection of public: Very few transnational groups have pursued a BW program, but the ones that did intended to kill Americans (see Section 16.3 through 16.6). Only three transnational terrorist groups (Al Qaeda Central, Jemaah Islamiyah, and Aum Shinrikyo), have sought a biological weapons capability based on open source reporting. Section 16.4 and Section 16.6 account for other lesser activities (such as empty threats of use, or the use of biological waste to spike explosive shrapnel) and "false positives" (groups that were initially believed to have sought BW, but where a reassessment of the evidence has disputed the prior accounts). Of these three transnational groups, only Al Qaeda is currently likely pursuing a biological weapons capability. Jemaah Islamiyah's membership, including its core leadership and all known BW-program members, has been decimated in recent years. Aum Shinrikyo's WMD program has been dismantled.

Furthermore, no credible open source reports exist that would indicate that new groups, such as ISIL or the al-Nusra Front, are attempting to obtain pathogens for use as biological weapons (see Section 16.3 and 16.4 on terrorist interest in BW, and Section 16.12 on ISIL).

Published studies that attempt to predict which future groups are most likely to pursue BW have produced widely varying results, indicating that no credible method exists to predict future terrorist interest in a BW capability. These efforts can be divided into two categories: quantitative searches for predictive indicators, and more qualitative organizational learning studies. The aforementioned quantitative study analyzing a dataset of 395 terrorist organizations active in the 1998–2005 period showed that only 23 had reportedly pursued some type of CBRN capability.[2252] The authors concluded that the larger the organization, the greater the number of attacks of any type it had previously launched, and the greater the number of allied groups the organization had, the more likely the organization was to pursue chemical, biological, radiological or nuclear (CBRN) weapons.[2253] They further noted that the ideologies of groups interested in CBRN weapons varied widely and reported that in particular, religious ideology was not a significant predictor for whether or not a group would seek CBRN weapons.[2254] Based on these findings, the authors noted "the apparent ascendancy of organizational variables (alliance connections, inexperience, and to [a] less certain extent, organizational size) over other factors, such as the much touted influence of religion."[2255] However, quantitatively determining whether any of these conclusions hold true when only biological weapons-seeking groups are analyzed is difficult, given the tiny sample size of groups who have sought such a capability. For example, one profile-based predictive model quantifying the risk of certain groups launching CBRN attacks failed to identify Jemaah Islamiyah as interested in BW; the model predicted a less than two percent chance that the group would pursue CBRN-type attacks.[2256] One study attempted to combine quantitative work with qualitative studies in part to move beyond this limitation. This 2014 study conducted by START researchers Gary Ackerman and Markus Binder used three different methods to generate a "top ten" list of current biological non-state adversaries.[2257] Using a new self-created dataset, they generated a quantitative model and produced a first ranking.[2258] This list

[2252] Ibid.
[2253] Ibid.
[2254] Ibid.
[2255] Ibid.
[2256] Alexandra Pocek Joosse, H. Brinton Milward, "Organizational Versus Individual Attribution: A Case Study of Jemaah Islamiyah and the Anthrax Plot," *Studies in Conflict & Terrorism* 37 (2014): p. 237, p. 253fn.4.
[2257] Gary Ackerman, Markus Binder (for START), "Anatomizing the Behavior of Chemical and Biological Non-State Adversaries," PASCC Semi-Annual Workshop on Strategic Stability and WMD, Washington, U.S.A., December 5, 2014, p. 11, http://csis.org/files/attachments/141205_Ackerman_Slides_0.pdf. Accessed July 13, 2015.
[2258] Ibid.

was then compared to results from their own assessment based on the historical cases in their dataset, as well as rankings provided by external subject matter experts.[2259] The resultant three lists differed widely from one another. In conclusion, no rigorous method exists for identifying terrorist groups who have not yet been caught pursuing BW but who are likely to do so in the future.

Although determining whether the group will pursue a BW program in the future is difficult, ISIL is of particular concern given its enormous resources, the fact that other groups abroad have sworn fealty to the organization, its ability to recruit or coerce engineers and scientists both in Syria and Iraq and in Western countries, its anti-US rhetoric and actions, its predilection for carrying out atrocities that it knows will generate mass media attention, its apocalyptic beliefs, and its apparent use of chemical weapons. As of August 2015, ISIL had already carried out or inspired 55 plots against the West (including Australia) and was linked to 14 plots against the US in particular.[2260] Section 16.12 summarizes relevant information on the group.

16.5.5.10 Deliberate Self-Infection

No such cases were uncovered in open source reporting. The motive for a deliberate self-infection by a transnational terrorist member would most likely be limited to infecting others. As explained in the section above, only three transnational terrorist groups have sought to infect others. None are known in open sources to have planned for self-infection as a means of doing so (see Section 16.3 and 16.4 on terrorist interest in BW, and Section 16.12 on ISIL).[2261]

16.5.6 Assessment of Malicious Acts Options for Foreign Intelligence Entities

16.5.6.1 Armed Assault, Bombing or Arson, Sabotage

A number of foreign intelligence agencies have the capability to orchestrate an armed assault against a US lab, or a bombing or an arson attack against a US lab, or the covert or overt sabotage of a US lab. However, such a direct act would be cause for war and, therefore, be highly unlikely.

16.5.6.2 Covert Entry (Physical)

No instances of physical covert entry into a US biology lab by an individual or team sent by a foreign intelligence agency were found in open source reporting, although this event is not outside the realm of possibility given that foreign intelligence agencies have targeted US labs before to steal research information. Indeed, this type of incident is unlikely to be captured in open sources, especially as successful covert entries may go entirely undetected. However, the subversion of an employee or the use of a cyber-espionage tool is significantly easier to organize from afar, and are, therefore, probably the preferred options. Reflecting on the differences between a cyber-espionage campaign and the recruitment and handling of an agent for espionage, the Office of the National Counterintelligence Executive noted

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[2259] Ibid.

[2260] Chairman Michael McCaul, Committee on Homeland Security, "Chairman McCaul Releases August 'Terror Threat Snapshot'," August 4, 2015, http://homeland.house.gov/press-release/chairman-mccaul-august-terror-threat-snapshot, http://homeland.house.gov/sites/homeland.house.gov/files/documents/August%20Terror%20Snapshot_0.pdf. Accessed August 11, 2015.

[2261] Note that terrorist discussion of BW carried out through "martyrdom" (suicide) operations does not imply self-infection as the chosen vector of spread.

that cyber-espionage was "faster and cheaper" and that it solved the logistical problem of having to transfer large volumes of documents from an agent to their foreign handler.[2262]

16.5.6.3 Covert Entry (Cyber)

A number of cyber-espionage campaigns have been mounted in recent years that have apparently targeted, *inter alia*, US research institutions and biopharmaceutical industries. Several such campaigns were persistent, well-organized, and appeared state-sponsored. Sophisticated cyber-espionage campaigns that have targeted at least in part the pharmaceutical industry include the "Epic Turla" and "Dragonfly" cyber-campaigns.[2263,2264] The "Epic Turla" campaign saw the infection of "several hundred" computers across 45 countries, including those of "government institutions, embassies, military, education, research and pharmaceutical companies."[2265] These incidents raise the possibility that laboratory research could be stolen by a foreign state or by a criminal group working for a foreign state without the need to carry out a physical covert entry operation. The "Dragonfly" campaign apparently targeted industrial control devices controlling pharmaceutical production lines to gain access to sensitive information and potentially steal "proprietary recipes and production batch sequence steps, as well as [...] information that indicate manufacturing plant volumes and capabilities."[2266] The subversion of pharmaceutical industrial control systems raises the possibility of plant sabotage and not just espionage, although the "Dragonfly" campaign did not involve sabotage operations.[2267]

16.5.6.4 Theft of Pathogens

Whether actual pathogen samples, rather than research material, were exfiltrated out of US laboratories by foreign agents remains unknown.

16.5.6.5 Theft of Material or Information

Theft of research material has occurred previously at US biology labs. The Soviet Union's KGB Directorate T of its First Main Directorate conducted scientific espionage against the West.[2268] KGB agents in the 1980s were tasked to report on select pathogen and toxin research, including influenza, in addition to other information. KGB agent handlers were especially interested in the "presence and characteristics of microorganisms with altered properties (new strains resistant to drugs and to the action of chemical and physical environmental factors, not detectable by standard serodiagnostic methods, carrying genetic determinants of virulence of heterogeneous microbial species, and capable of overcoming specific immunity)."[2269] Targets for information collection included the National Institutes of Health, selected due to its research on chemical and biological warfare agent effects.[2270] KGB archives

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2262 Office of the National Counterintelligence Executive, "Report to Congress on Foreign Economic Collection and Industrial Espionage, 2009-2011," p.2.

2263 Kaspersky Lab Global Research and Analysis Team, "The Epic Turla Operation: Solving some of the mysteries of Snake/Uroburos," *SecureList*, August 7, 2014, https://securelist.com/analysis/publications/65545/the-epic-turla-operation/ Accessed July 14, 2015.

2264 Kaspersky Lab Global Research and Analysis Team, "Energetic Bear – Crouching Yeti," July 31, 2014, p. 2, https://securelist.com/files/2014/07/EB-YetiJuly2014-Public.pdf. Accessed July 14, 2015.

2265 Kaspersky Lab Global Research and Analysis Team, "The Epic Turla Operation: Solving some of the mysteries of Snake/Uroburos."

2266 Joel T. Langill, "Defending Against the Dragonfly Cyber Security Attacks," version 3.0, White Paper, Belden, December 10, 2014, p. 1, 5-8.

2267 Ibid.

2268 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press

2269 Ibid.

2270 Christopher Andrew, Vasili Mitrokhin, *The Sword and the Shield: The Mitrokhin Archive and the Secret History of the KGB* (New York: Basic Books, 1999), p. 614 fn. 109.

exfiltrated by Mitrokhin reportedly showed the presence of a KGB agent at the conglomerate Du Pont de Nemours, which conducted work in the chemical, petrochemical, and biomedical sectors.[2271] Experts believe that this collection effort was at least in part conducted in support of the covert Soviet BW program.[2272]

The theft of biotechnology and research on pathogens for commercial gain or for unknown purposes by state actors is believed to be ongoing. One example of an alleged attempted physical theft occurring in a laboratory outside of the US was reported by Russian Federal Security Service (FSB) officers, who stated on December 22, 2004 that they had prevented an attempt by foreign intelligence to extract research data from the Russian biological research institute VECTOR.[2273] VECTOR is an institute that conducts research on extremely dangerous pathogens, and is one of the two official repositories of the smallpox virus.[2274] The regional head of the FSB, Sergei Savchenkov, further remarked that foreign agents had been specifically targeting microbiology and genetic engineering research.[2275] Whether these claims are true or propaganda is unclear.

16.5.6.6 Elicitation of Information

Elicitation for espionage purposes has certainly occurred, for instance in support of the aforementioned KGB information campaigns. Elicitation incidents are however rarely documented in open sources, as a successful elicitation will not raise the suspicion of the victim and hence is likely to go by unreported.[2276]

16.5.6.7 Subversion of Employee

Employees at US labs have been subverted by foreign intelligence agencies previously, as the aforementioned KGB cases demonstrate. The KGB office in the United Kingdom (UK) had for their part recruited a lab assistant in the UK under the code name STEP.[2277]

16.5.6.8 Insertion of operative

As in the case of "covert entry (physical)," no such instances were uncovered in open source reporting, although the possibility of the insertion of an operative into a US lab cannot be ruled out. The subversion of an employee is by far easier to organize from abroad and, along with cyber-espionage, probably one of the preferred options.

16.5.6.9 Reckless Act…

Given the rigorous vetting process used by intelligence agencies on their employees, most reckless acts by a foreign agent or foreign agent team infiltrated into a laboratory can be dismissed. In cases where an individual is recruited by a foreign intelligence agency in country, the risks of reckless acts may be similar to an act carried out by a lone insider.

2271 Ibid

2272 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.

2273 IHS Jane's, "RIA-Novosti news agency reported on …" *Jane's Intelligence Watch Report - Daily Update*, December 23, 2004.

2274 World Health Organization. "World Health Organization inspects Russian smallpox laboratory," October 25, 2002, http://www.who.int/mediacentre/news/notes/np7/en/. Accessed August 11, 2015.

2275 IHS Jane's, "RIA-Novosti news agency reported on …"

2276 Federal Bureau of Investigation (FBI), "Elicitation Technique."

2277 Ibid.

... *Infection of outside animals (Wild or domestic):* Foreign intelligence agencies might hypothetically be tasked by an unscrupulous government to covertly infect animals to cause serious economic damage to a rival state.[2278] Despite a number of allegations, no modern cases have been confirmed in open source reporting. The only confirmed case of anti-animal warfare by a foreign state is Germany's covert anti-animal BW operations in World War I, which targeted pack animals (horses in the United States) in an attempt at disrupting war logistics.[2279,2280,2281]

... *Infection of public:* A state willing to violate its international obligations under the 1972 Biological Weapons Convention and international customary norms could hypothetically attempt to weaponize a pathogen obtained from a laboratory. A number of foreign intelligence agencies, including the Soviet Union's and Apartheid South Africa's, have historically considered using biological weapons for assassination purposes, i.e., against the public, but in practice have relied on chemical poisons (including toxins).[2282,2283,2284,2285]

No useable weapon based on influenza is known to have been developed, and modern programs run by foreign states (covertly, in contravention to the BWC) are not known to include weaponization influenza virus strains.[2286] However, a US Department of Defense planning document on responding to pandemic influenza prepared a question-and-answer segment that summarized the arguments as follows: "many strains of influenza have significant potential for bioterrorism," but "because the flu virus mutates so easily many other organisms would be a better and easier choice for an aggressor to use."[2287] The relatively rapid mutation rate of influenza is a recognized barrier to hypothetical weaponization.[2288]

2278 Piers Millett, "Antianimal Biological Weapons Program," *Deadly Cultures: Biological Weapons Since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Harvard University Press, 2006), p. 233-235.

2279 France may have reciprocated during WWI by infecting horses destined to Germany, but additional research on the topic is necessary. W. Seth Carus, "The History of Biological Weapons Use: What We Know and What We Don't," *Health Security* 13, no. 4 (2015): p 233-234.

2280 Mark Wheelis, "Biological sabotage in World War I," *Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945, Chemical & Biological Warfare Studies No. 18*, eds. Erhard Geissler, John Ellis van Courtland Moon (Oxford University Press, 1999), p. 35-59.

2281 See also the following study, which looked at incidents in North America only: G. A. Ackerman, J. Giroux, "A history of biological disasters of animal origin in North America," *Scientific and Technical Review of the Office International des Epizooties (Paris)* 25, no. 1 (2006): p. 87.

2282 For example, the Soviet Union considered the use of a *Y. pestis* dispenser to assassinate Yugoslav leader Josip Tito until Stalin's death in 1953 put a halt to the assassination planning. "Stalin's Plan to Assassinate Tito," Cold War International History Project Bulletin 10, March 1998, p. 137. In the post-BWC covert Biopreparat period, the KGB ran a program called Flute that studied biological weapons suitable for assassinations, notably bioregulatory peptides.

2283 On the Apartheid South African program, see: Chandré Gould, Alastair Hay, "The South African Biological Weapons Program", Stephen Burgess, Helen Purkitt, *The Rollback of South Africa's Chemical and Biological Warfare Program*, USAF Counterproliferation Center, April 2001, p. 8-9, 13-16, 21, http://www.au.af.mil/au/awc/awcgate/cpc-pubs/southafrica.pdf. Accessed September 16, 2015.

2284 On Iraq's intelligence services and their clandestine labs, see the comprehensive CIA report based on investigations following the 2003 Iraq War: Central Intelligence Agency (CIA), "DCI Special Advisor Report on Iraq's WMD, Volume I Iraq's Intelligence Services," September 30, 2004, https://www.cia.gov/library/reports/general-reports-1/iraq_wmd_2004/chap1_annxB.html#sect7. Accessed September 16, 2015.

2285 Central Intelligence Agency (CIA), "DCI Special Advisor Report on Iraq's WMD, Volume 3: Biological Warfare," September 30, 2004, https://www.cia.gov/library/reports/general-reports-1/iraq_wmd_2004/chap6.html#sect4. Accessed September 16, 2015.

2286 Yannick Pouliot, Jennifer L. O. Sheer, "Influenza," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 322-323.

2287 United States Northern Command (USNORTHCOM) Concept of Operations Plan (CONPLAN) 3551-09, Concept Plan to Synchronize DOD Pandemic Influenza Planning. August 13, 2009, Released under the Freedom of Information Act on June 27, 2013, F-1-3, retrieved at http://www.governmentattic.org/8docs/NORTHCON_CONPLAN_3551-09_2009.pdf. Accessed July 14, 2015.

2288 Ibid.

The defunct and pre-BWC Canadian offensive BW program studied influenza A and B in the 1960s.[2289] The Canadian BW work was coordinated with the defunct and pre-BWC US and UK offensive BW programs as part of the Tripartite Alliance.[2290] Influenza A was recommended as a field trial agent because it was "a relatively mild disease in man though it may be temporarily debilitating […] [it] is dangerous only to the very young and the aged and then only as a result of secondary bacterial infection."[2291] The defunct and pre-BWC French offensive BW program considered influenza virus A/PR/8 in 1966 as part of its study on biological incapacitants (the results and conclusions of this initial study are unknown).[2292] These initial studies did not lead to useable weapons.

The US military appears to have conducted research on human-transmissible influenza for defensive purposes, particularly for preventing infection of US armed forces with naturally-occurring influenza.[2293] In addition, the US military had concerns that influenza could be used against US forces. For instance, in June 1961, Colonel Tigertt listed the flu as one out of 40 potential diseases that could be unleashed as part of biological warfare.[2294] The US military conducted volunteer human testing with influenza virus, but influenza was only one of a long list of potential BW agents assessed.[2295] Finally, the US military screened bovine and avian influenza virus strains for potential as anti-animal warfare agents. Fort Terry (1952-1954) screened for, researched, and developed certain anti-animal biological warfare agents in its brief existence (1952-1954).[2296] The site held one bovine influenza strain and 34 avian influenza strains.[2297] Avian influenza was selected for its weapons potential, and classed as a second-tier agent in a three-tier ranking system.[2298] However, by the time of the United States' 1969 unilateral renunciation of biological weapons, no influenza-based weapon was in US stockpiles, as evidenced by now-declassified documents on materials to be destroyed to comply with the renunciation announcement.[2299] Indeed, the official US Army history of the US BW program makes no mention of any influenza virus-based weapons, nor of any influenza virus production lines, nor of any field tests involving influenza virus.[2300],[2301]

2289 Donald Avery, "The Canadian Biological Weapons Program and the Tripartite Alliance," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p.405fn.49.

2290 Donald H. Avery, *Pathogens for War: Biological Weapons, Canadian Life Scientists, and North American Biodefense* (Toronto: University of Toronto Press, 2013), p.113.

2291 Ibid.

2292 Olivier Lepick, "The French Biological Weapons Program," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p. 125.

2293 Dan Crozier, "History of the Commission on Epidemiological Survey," Section 1 Part IV, The Armed Forces Epidemiological Board: The Histories of the Commissions, ed. Theodore E. Woodward (Washington: Borden Institute, 1994), p. 91, 111, 150, 153.. Retrieved at: U.S. Army Medical Department, Office of Medical History, "The Histories of the Commissions – Contents," http://history.amedd.army.mil/booksdocs/historiesofcomsn/commission.html. Accessed July 14, 2015.

2294 Ibid, p. 237.

2295 John Ellis van Courtland Moon, "The U.S. Biological Weapons Program," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p. 26.

2296 Piers Millett, "Antianimal Biological Weapons Program," *Deadly Cultures: Biological Weapons Since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Harvard University Press, 2006), p. 226.

2297 Ibid.

2298 Ibid.

2299 See Tab A: Material to be Destroyed (Biological and Toxin), in:
The Secretary of Defense, "Memorandum For the President, National Security Decision Memoranda 35 and 44," July 6, 1970, Declassified, p.3, http://nsarchive.gwu.edu/NSAEBB/NSAEBB58/RNCBW22.pdf. Accessed June 30, 2015.

2300 U.S. Department of the Army, "U.S. Army Activity in the U.S. Biological Warfare Programs, Volume 1," p. 50-51.

2301 U.S. Department of the Army, "U.S. Army Activity in the U.S. Biological Warfare Programs, 1942-1977, Volume 2," February 24, 1977, Unclassified, p. 102, 124-140, http://nsarchive.gwu.edu/NSAEBB/NSAEBB58/RNCBW_USABWP.pdf. Accessed June 30, 2015.

Post-1972 offensive BW programs (and therefore post-BWC, covert, programs) apparently did not include influenza for weaponization. Apartheid South Africa, Iraq, and the Soviet Union ran offensive BW programs of widely different scale and degree of sophistication in this timeframe.[2302,2303,2304,2305,2306] None are known to have selected influenza as a weapons pathogen, even though the Soviet Union apparently believed that influenza ("classical fowl plague") might be weaponizable, since it remained on a long list of pathogens that KGB agents were supposed to monitor from Western research.[2307,2308,2309,2310]

16.6 Attacks Against Laboratories

Numerous confirmed cases of attacks against research and medical laboratories have occurred in the US and abroad, including one operated by a foreign ministry of defense. Eighteen cases between 1990 and 2015 were found in open source literature and documented in Table 16.1. In addition, two cases from 1989 and 1987, which involved the theft of an infected animals, are documented in Table 16.1 because they are relevant for assessing potential malicious actor motivations and capabilities, and malicious acts. Laboratory security increased in the 1990s, in part in response to prior incidents. These increases were highlighted by supporters of the Animal Liberation Front (ALF), a decentralized domestic extremist group that was responsible for most of the incidents documented below.[2311]

2302 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.

2303 United Nations, S/1995/864, October 11, 1995, http://www.un.org/Depts/unscom/sres95-864.htm. Accessed September 27, 2015.

2304 Graham S. Pearson, "The Iraqi Biological Weapons Program," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p. 177-179, 181.

2305 United Nations Monitoring, Verification and Inspection Commission (UNMOVIC), "Compendium: Chapter V: The Biological Weapons Programme," p. 766-1030.
http://www.un.org/Depts/unmovic/new/documents/compendium/Chapter_V.pdf. [Dead link]

2306 Chandré Gould, Alastair Hay, "The South African Biological Weapons Program," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p. 197-200.

2307 Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.
United Nations Monitoring, Verification and Inspection Commission (UNMOVIC), "Compendium: Chapter V: The Biological Weapons Programme," p. 766-1030.

2308 United Nations, S/1995/864.

2309 Graham S. Pearson, "The Iraqi Biological Weapons Program," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p. 177-179, 181.

2310 Chandré Gould, Alastair Hay, "The South African Biological Weapons Program," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p. 197-200.

2311 See the following article on a website maintained in support of the Animal Liberation Front: "Laboratory Animal Liberation Campaign," Animal Liberation Front, http://www.animalliberationfront.com/ALFront/lab.htm. Accessed August 11, 2015.

Table 16.1. Known Attacks Against Research and Medical Laboratories, 1989–2015	
2013-12-05	A suicide car bombing breached a Ministry of Defense complex in Yemen, enabling gunmen to enter and attack the Al-Oradi military hospital and laboratory. Al Qaeda in the Arabian Peninsula (AQAP) claimed responsibility.[2312]
2013-04-22	Animal rights extremists broke into a laboratory in Milan, Italy.[2313] They released mice and rabbits, and switched cage labels to sabotage research.[2314] According to a worker at the facility, "The lack of signs of a break-in suggests that the activists may have used an illegally acquired electronic card."[2315] Two members chained themselves to the main double doors. As a result of negotiations with authorities, the group members were allowed to leave with "fewer than 100 animals."[2316]
2012-02-28	A bomb planted against a Pakistani university building damaged, *inter alia*, a laboratory.[2317]
2011-08-28	A bomb attack against a Pakistani hospital destroyed a medical laboratory.[2318]
2008-02-01	The Biomedical Research Institute of the University of Hasselt (Belgium) was set ablaze.[2319][2320]
2005-04-22	Members of the Animal Liberation Front (ALF) claimed responsibility for vandalizing a biology laboratory at the Louisiana State University (US) The perpetrators exfiltrated 10 mice, stole some cages, moved mice from cages to cages, and removed mice cage identifying cards.[2321] Overall, members of the ALF had access to a little over 80 mice being used in experiments.[2322]

[2312] Indexed in the START Global Terrorism Database, GTD ID: 201312050016, http://apps.start.umd.edu/gtd/search/IncidentSummary.aspx?gtdid=201312050016. Accessed June 29, 2015.

[2313] Alison Abbott, "Animal-Rights activists wreak havoc in Milan laboratory," *Nature*, April 22, 2013, http://www.nature.com/news/animal-rights-activists-wreak-havoc-in-milan-laboratory-1.12847. Accessed October 15, 2015.

[2314] Ibid.

[2315] Ibid.

[2316] Ibid.

[2317] "University campus blown up in Charsadda," *Dawn*, March 1, 2012, http://www.dawn.com/news/699336/university-campus-blown-up-in-charsadda-2. Accessed June 29, 2015. Indexed in the START Global Terrorism Database, GTD ID: 201202280031, http://apps.start.umd.edu/gtd/search/IncidentSummary.aspx?gtdid=201202280031. Accessed June 29, 2015.

[2318] Attack mentioned in: "Girls school, shops blown up in Swabi," *Dawn*, August 30, 2011, http://www.dailytimes.com.pk/default.asp?page=2011\08\30\story_30-8-2011_pg7_21 [Dead link]. Indexed in the START Global Terrorism Database, GTD ID: 201108280002, http://apps.start.umd.edu/gtd/search/IncidentSummary.aspx?gtdid=201108280002. Accessed June 29, 2015.

[2319] Geoff Brumfiel, "Animal-rights activists invade Europe," *Nature* (News) 451 (2008): p.1034-1035, http://www.nature.com/news/2008/080227/full/4511034a.html. Accessed July 17, 2015.

[2320] TVL Limburg, archived page at: https://web.archive.org/web/20080208121228/http://www.tvl.be/nl/nieuws/2008-02-03/brandstichting-alf-op-luc/. Accessed June 29, 2015.

[2321] Statement of Senator David Vitter, "opening statement," Oversight on Eco-terrorism specifically examining the Earth Liberation Front ("ELF") and the Animal Liberation Front ("ALF"), U.S. Senate Committee on Environment & Public Works, May 18, 2005, http://www.epw.senate.gov/pressitem.cfm?party=rep&id=237834, http://www.epw.senate.gov/hearing_statements.cfm?id=237836. Accessed July 13, 2015.

[2322] Ibid.

Table 16.1. Known Attacks Against Research and Medical Laboratories, 1989–2015	
2004-11-14	Two laboratories at the University of Iowa (US) were vandalized, including through the pouring of chemicals, and 401 rats and mice were exfiltrated.[2323,2324] Members of the ALF claimed responsibility. Video footage released by the group showed the perpetrators had electronic keys, facilitating access.[2325]
2003-12-13	695 mice were exfiltrated out of Wickham Laboratories (UK) by two members of the ALF. The laboratory held botulinum toxin in the form of Dysport, which is a product used for therapeutic purposes.[2326]
2003-09-24	Members of the ALF destroyed equipment at the Louisiana State University Inhalation Toxicology Research Facility at the School of Veterinarian Medicine (US).[2327,2328]
2001-11-12	Acid and bleach were poured throughout the Sierra Biomedical (US) research facility. Members of the ALF claimed responsibility.[2329]
2001-09-20	An incendiary device went off at the White Sands Research Center (US) a laboratory which used chimpanzees for medical testing. Members of the ALF claimed responsibility.[2330]
1999-12-31	The Agricultural Hall of the University of Michigan State University was destroyed by arson (accelerant was found at the scene).[2331] Members of the ELF claimed responsibility.[2332] The attack was launched ostensibly in opposition to a crop genetic engineering research project, but the Agricultural Hall itself was not a research laboratory and only contained research data on the project.[2333,2334]
1999-11-19 or 20	Animal rights extremists broke into the Avian Health Laboratory of the Washington State University, destroyed equipment, and poured chlorine throughout the facility.[2335]

[2323] David Frabotta, "Vandals upend University of Iowa lab," *DVM360 Magazine*, January 1, 2005, http://veterinarynews.dvm360.com/vandals-upend-university-iowa-lab. Accessed June 29, 2015.

[2324] Ann McGlynn, "Activist who refused grand jury testimony now charged with conspiracy," *Lancaster Online*, November 19, 2009, http://lancasteronline.com/your_news/community/activist-who-refused-grand-jury-testimony-now-charged-with-conspiracy/article_3e187816-29c4-5c46-89aa-6edfbf8c8cfc.html?mode=jqm. Accessed July 13, 2015.

[2325] Ann McGlynn, "Activist who refused grand jury testimony now charged with conspiracy."

[2326] "Veteran animal rights activist jailed after threat in court," *The Guardian*, April 30, 2005, http://www.theguardian.com/uk/2005/apr/30/businessofresearch.animalwelfare. Accessed June 29, 2015.

[2327] R. Scott Nolen, "LSU laboratory vandalized; animal extremist group claims responsibility," *JAVMA News*, November 1, 2003, https://www.avma.org/News/JAVMANews/Pages/031101a.aspx. Accessed June 29, 2015.

[2328] Samantha Sieber, "FBI investigates Vet School break-in," *LSU Reveille*, September 25, 2003, http://www.lsureveille.com/fbi-investigates-vet-school-break-in/article_72954388-993b-5498-9fbf-975a5fde5e4f.html. Accessed June 29, 2015.

[2329] Federal Bureau of Investigation (FBI), "Terrorism 2000/2001," http://www.fbi.gov/stats-services/publications/terror/terrorism-2000-2001. Accessed June 29, 2015.

[2330] Indexed in the START Global Terrorism Database, GTD ID: 200109200006, http://apps.start.umd.edu/gtd/search/IncidentSummary.aspx?gtdid=200109200006. Accessed June 29, 2015.

[2331] Federal Bureau of Investigation (FBI), "Terrorism in the United States 1999," p. 6, https://www.fbi.gov/stats-services/publications/terror_99.pdf. Accessed August 28, 2015.

[2332] Ibid.

[2333] Ibid.

[2334] "Four Arrested in 1999 New Year's Eve Agriculture Hall arson," *MSU Special Report*, March 11, 2008, retrieved at https://web.archive.org/web/20141025083650/http://special.news.msu.edu/ag_hall/index.php. Accessed August 27, 2015.

[2335] Mark Rahner, "Equipment is Destroyed at WSU Research Center- Animal Liberation Front Claims Responsibility," *The Seattle Times*, November 22, 1999, http://community.seattletimes.nwsource.com/archive/?date=19991122&slug=2996770 Accessed October 15, 2015.

Table 16.1. Known Attacks Against Research and Medical Laboratories, 1989–2015	
1999-10-23 or 24	Animal rights extremists into a laboratory in Bellingham run by Western Washington University. Four rabbits and 37 white rats were exfiltrated.[2336] The perpetrators tried to break into the room where non-human primates were kept, but failed.[2337]
1999-08-28 and 29	Members of the ALF broke into a laboratory owned by private biotechnology company Bio-Devices in Orange County, California and exfiltrated 55 dogs.[2338] Lead X-ray gowns and bottles of medication were stuffed into the facility's sinks "in an apparent attempt to cause flooding."[2339]
1999-04-05	Members of the ALF broke into twelve laboratories at the University of Minnesota in one night, causing $2 million in damages and exfiltrating some 100 research animals (pigeons, rats, and mice).[2340] Some of the animals were later found dead in a field, having been abandoned.[2341] One media account provides specific details regarding the break-in at researcher Walter Low's laboratory.[2342] The ALF members poured chemicals on equipment and papers, and destroyed microscopes, computers, and a particularly valuable tissue culture.[2343] However, they failed to break into the mice storage room in this particular laboratory.[2344]
1992-02-28	Members of the ALF broke into two animal research buildings on the Michigan State University campus (US) and set fire to offices, poured sulfuric acid into laboratory equipment, and opened the cages of minks held for research (the animals did not escape).[2345]
1991-08-12 or 13	Members of the ALF broke into two office buildings and released seven coyotes, 10 mice, and six minks from two animal research facilities at the Washington State University.[2346][2347] A media report at the time noted that one of the offices broken into had no signs of forced entry, and that police was wondering whether a key had been used.[2348]

[2336] Janet Burkitt, "Research Animals Taken From Laboratory- Police Suspects Activists Involved in Wwu Break-in," *The Seattle Times*, October 25, 1999, http://community.seattletimes.nwsource.com/archive/?date=19991025&slug=2991001. Accessed October 15, 2015.
[2337] Ibid.
[2338] Federal Bureau of Investigation (FBI), "Terrorism in the United States 1999," p. 5.
[2339] Ibid.
[2340] Howard Bell, "Of Mice and Medicine," *Minnesota Medicine*, April 2007, http://www.minnesotamedicine.com/Past-Issues/Past-Issues-2007/April-2007/Feature-April-2007. Accessed August 3, 2015.
[2341] Ibid.
[2342] Ibid.
[2343] Ibid.
[2344] Ibid.
[2345] "Animal Rights Raiders Destroy Years of Work," *The New York Times*, March 8, 1992, http://www.nytimes.com/1992/03/08/nyregion/campus-life-michigan-state-animal-rights-raiders-destroy-years-of-work.html. Accessed June 29, 2015.
[2346] Eric Sorensen, "Activists vandalize WSU labs, release research animals," *The Spokesman-Review*, A1, A7, retrieved at: Animal Liberation Frontline, Animal Liberation Front Press Clippings 1984-1994, p.20-21, http://animalliberationfrontline.com/wp-content/uploads/2014/10/ALF-News-Article-Collection.pdf.
[2347] Mark Rahner, "Equipment is Destroyed at WSU Research Center- Animal Liberation Front Claims Responsibility."
[2348] Eric Sorensen, "Activists vandalize WSU labs, release research animals," A7.

Table 16.1. Known Attacks Against Research and Medical Laboratories, 1989–2015	
1989-04-03	Members of the ALF claimed responsibility for the arson of two research buildings, including a diagnostic laboratory, at the University of Arizona (US) They claimed to have released over 1000 animals from three research facilities. A researcher working at the facility noted that 30 missing mice were infected at the time of release with cryptosporidium.[2349] The perpetrators claimed in a press release that "absolutely no animals were released into the community." that "all animals were carefully transported to safe houses," and that "the infected mice were [...] being treated."[2350]
1987-08-23	An animal rights extremist group, the Band of Mercy, broke into the Beltsville Agricultural Research Center in Maryland by cutting through a six-foot link fence and breaking padlocks.[2351] The laboratory was run by the Department of Agriculture and the break-in resulted in an FBI investigation.[2352] The group members exfiltrated seven African miniature pigs, as well as 28 cats.[2353] Of these animals, eleven of the cats were infected with *Toxoplasma gondii* as part of an experiment.[2354] The theft was discovered early morning on Sunday, August 23, 1987.[2355] The members reportedly knew that the cats were infected at the time of the theft, but the group reportedly gave assurances that the cats had been put under veterinary care after the break-in.[2356]

16.7 Biocrimes Committed by Individuals

A "biocrime" is defined here as a criminal act, excluding terrorism, involving a biological substance as follows: a pathogen, a genetic construct thereof, and medical waste when used with the intent to threaten infection. Hoaxes and "empty" threats where the actor did not possess or could not be demonstrated to have possessed a biological substance are not included in this assessment.[2357] Incidents involving toxins such as ricin, which are chemicals, are not included in this assessment. Terrorist incidents, including lone operator terrorism, are not included in this annex, but are addressed in the terrorism incident annex.

The table below is a compilation of biocrimes, drawing from existing collections in the literature:

- W. Seth Carus' *Bioterrorism and Biocrimes*, which documented a total of 16 confirmed biocrimes (excluding terrorism) where the perpetrator acquired and used a biological agent and another seven that had acquired an agent, from 1900 to 1999;[2358]

2349 "Diseased mice freed in arson fires, break-in," *Spartanburg Herald-Journal*, April 4, 1989, A2. Retrieved at: https://news.google.com/newspapers?nid=1876&dat=19890404&id=2kwsAAAAIBAJ&sjid=Vs4EAAAAIBAJ&pg=6664,1859692&hl=en. Accessed June 29, 2015.

2350 Animal Liberation Frontline, Animal Liberation Front Press Clippings 1984-1994, p.29, http://animalliberationfrontline.com/wp-content/uploads/2014/10/ALF-News-Article-Collection.pdf. Accessed October 15, 2015.

2351 Keith Schneider, "Theft of Infected Cats From U.S. Lab Spurs Alert," *The New York Times*, August 25, 1987, http://www.nytimes.com/1987/08/25/us/theft-of-infected-cats-from-us-lab-spurs-alert.html. Accessed October 15, 2015.

2352 Ibid.

2353 Ibid.

2354 Ibid.

2355 Ibid.

2356 As given in an account "intended to represent the views of the United States Animal Liberation Front and its members." Ingrid Newkirk, *Free the Animals: The Amazing True Story of the Animal Liberation Front* (New York: Lantern Books, 2000). p. 339-355, front matter, and pictures.

2357 These discarded cases are primarily *B. anthracis* letter hoaxes and cases where individuals threatened others with what they claimed were "HIV-infected" sharp objects but where no evidence of actual HIV contamination was found.

2358 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, Working Paper (Washington: Center for Counterproliferation Research, National Defense University, February 2001 Revision), p. 8.

- A short list of incidents compiled by FBI for a 2013 AAAS – AAU – APLU – FBI report on personnel security programs,[2359] and

- A synthesis list of CBRN incidents from 1950–2005, listing incidents found in all existing unclassified incident databases at the time, prepared by Hamid Mohtadi and Antu Murshid.[2360]

The cases included below are incidents that were reported as "confirmed" in the literature. For all events, an attempt was made to obtain the primary source material and verify the reported incident directly. When an incident was reported in open sources but was based solely on a primary source that was not publicly available, such as the CNS CBRN incident database or interview material reported by the National Research Council, the incident is reported even when it was not possible to verify the case. These incidents are clearly flagged as not yet resolved in the text.

The following incidents from 1990 to 2015 have been identified as biocrimes, although this list is unlikely to be complete. In particular, the coverage of foreign incidents is deficient and is limited to a handful of high-profile cases. In addition, several unconfirmed and unclear cases have been reported.[2361]

Table 16.2. Biocrimes, 1990 to 2015	
Charged 26 November 2014	Ouyang Xiangyu was a graduate student at Stanford University who allegedly sabotaged lab mates' research by killing off their stem cells, and then proceeded to attempt to poison lab mates and herself by putting paraformaldehyde in their water bottles as well as her own.[2362,2363] She pleaded not guilty due to insanity.[2364] The case is ongoing.[2365]
Charged 2012	David Kwiatkowski stole fentanyl syringes to feed his drug habit, and replaced the contents with a saline solution and his own contaminated blood.[2366] He was accused of infecting over 40 hospital patients with Hepatitis C, including one patient who subsequently died of the infection, and ended up sentenced to 39 years imprisonment.[2367]

2359 American Association for the Advancement of Science (AAAS), Association of American Universities (AAU), Association of Public and Land-grant Universities (APLU), Federal Bureau of Investigation (FBI), *Bridging Science and Security for Biological Research: Personnel Security Programs*, Meeting Report, Washington, United States, August 21-23, 2013, p. 37-42, http://www.aaas.org/sites/default/files/reports/AAAS-APLU-AAU-FBI%20report%20on%20personnel%20security%20070114.pdf. Accessed July 13, 2015.

2360 The databases in question were: the RAND-St. Andrews Terrorism Chronology, ITERATE, Pinkerton Corporation's Global Intelligence Service, the CNS WMD Database, and the MIPT Database. The authors relied on secondary literature compilations to access data from some of these databases.
Hamid Mohtadi, Antu Murshid, "A Global Chronology of Incidents of Chemical, Biological, Radioactive and Nuclear Attacks: 1950-2005," p. 1-5.

2361 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, Working Paper (Washington: Center for Counterproliferation Research, National Defense University, February 2001 Revision).

2362 Melissa Sim, "A*Star scholarship holder Ouyang Xiangyu expelled from Stanford," April 8, 2015, *The Straits Times*, http://www.straitstimes.com/singapore/courts-crime/astar-scholarship-holder-ouyang-xiangyu-expelled-from-stanford. Accessed September 10, 2015.

2363 "A*Star scholar charged for poisoning labmates' drinks," April 2, 2015, *TR Emeritus*, https://web.archive.org/web/20150404222631/http://www.tremeritus.com/2015/04/02/astar-scholar-charged-for-poisoning-labmates-drinks/. Accessed September 10, 2015.

2364 Ibid.

2365 Ibid.

2366 AAAS, AAU APLU, FBI, Bridging Science and Security for Biological Research: Personnel Security Programs, p. 40.

2367 Ibid.

Table 16.2. Biocrimes, 1990 to 2015	
July 1 and November 28, 2011	Mohsen Hosseinkhani was fired from his job as a cardiology post-doctoral fellow at the Mount Sinai Medical Center. He then broke into the laboratory on two occasions, stole equipment as well as "secret scientific material" (including stem cell cultures and antibodies), and sabotaged experiments by switching mice name tags.[2368,2369] He may have transported stolen materials to Russia.[2370,2371,2372] Once caught, he fled to Iran to avoid prosecution.[2373,2374]
Charged 2010	A post-doctorate student, Vipul Bhrigu, repeatedly sabotaged the work of another graduate student working at the same laboratory by spraying ethanol onto his victim's cell-culture media over several months.[2375,2376,2377] Once caught, Bhrigu confessed that he "got jealous of others moving ahead and [...] wanted to slow them down."[2378]
January 21, 2009 to May 5, 2009	Konan Michel Yao stole and attempted to smuggle into the US 22 vials containing DNA encoding Ebola genes taken from his prior employer, the National Microbiology Laboratory (Canada).[2379] He did so in an attempt to transfer his prior research to his new employer.[2380] He said he stole the vials on January 21, 2009, during his last day at the lab.[2381] He was then arrested at the US-Canada land border on May 5, 2009.[2382] The Canadian lab said they were reviewing their biosecurity protocol in response to this incident.[2383]
May 2007	An attempted theft "targeted at the pathogen collection at the central reference laboratory for animal health in Indonesia" was "thwarted by security systems installed by the US government."[2384] No further information has been released by the US Department of State.[2385] The motive behind the attempt, as well as whether this incident involved one or more individuals, is therefore unknown.

[2368] Anemona Hartocollis, Al Baker, "Doctor Accused of Crimes Against Mice and Lab," *New York Times – City Room Blog*, December 2, 2011, http://cityroom.blogs.nytimes.com/2011/12/02/doctor-accused-of-crimes-against-mice-and-lab/. Accessed July 13, 2015.

[2369] "Lab rat switcher jumps bail, flees to Iran," *Iran Times*, http://iran-times.com/lab-rat-switcher-jumps-bail-flees-to-iran/. Accessed July 13, 2015.

[2370] Anemona Hartocollis, Al Baker, "Doctor Accused of Crimes Against Mice and Lab."

[2371] Jamie Schram, "Doctor upset over losing hospital fellowship allegedly stole scientific materials, shuffled around lab rats," *New York Post*, December 2, 2011, http://nypost.com/2011/12/02/doctor-upset-over-losing-hospital-fellowship-allegedly-stole-scientific-materials-shuffled-around-lab-rats/. Accessed June 30, 2015.

[2372] AAAS, AAU APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 42.

[2373] Ibid.

[2374] "Lab rat switcher jumps bail, flees to Iran."

[2375] Bhrigu denied involvement in earlier cases of potential sabotage; the earliest event of potential sabotage flagged occurred in December 2009. Bhrigu stated that he had sabotaged his colleague's work starting in February 2010. Brendan Maher, "Research integrity: Sabotage!" *Nature (News)* 467, (2010): p. 516-518, http://www.nature.com/news/2010/100929/full/467516a.html. Accessed June 30, 2015.

[2376] Brendan Maher, "Lab sabotage deemed research misconduct (with exclusive surveillance video)," *Nature News Blog*, April 27, 2011, http://blogs.nature.com/news/2011/04/lab_sabotage_deemed_research_m_1.html. Accessed June 30, 2015.

[2377] AAAS, AAU APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 42.

[2378] Brendan Maher, "Research integrity: Sabotage!" p. 518.

[2379] AAAS, AAU APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 37.

[2380] Ibid.

[2381] "Winnipeg researcher charged with smuggling Ebola material into U.S.," *CBC News*, May 13, 2009, http://www.cbc.ca/news/canada/winnipeg-researcher-charged-with-smuggling-ebola-material-into-u-s-1.774725. Accessed June 30, 2015.

[2382] Ibid.

[2383] Ibid.

[2384] Committee on Prevention of Proliferation of Biological Weapons, Office for Central Europe and Eurasia, National Research Council, *The Biological Threat Reduction Program of the Department of Defense: From Foreign Assistance to Sustainable Partnership* (Washington: The National Academies Press, 2007), p.15, p.15 fn.4.

[2385] Ibid.

Table 16.2. Biocrimes, 1990 to 2015	
2002	A former Cornell University researcher named Qingqiang Yin attempted to smuggle to China over 250 vials, test tubes, and petri dishes in his luggage.[2386] Some containers were leaking when they were found.[2387] The samples are believed to have contained bacteria and yeast culture related to enzyme production work that the researcher had been part of.[2388] The researcher had recently been passed over for re-hire given his reported "poor job performance," and Yin was attempting to obtain a job at a laboratory in China.[2389]
June 18, 2000	The James Martin Center for Nonproliferation Studies (CNS) database holds a case regarding two Kuwaiti prison inmates threatened guards and other inmates with HIV-contaminated razor blades.[2390] CNS classified this as an "incident with possession," but no evidence was presented to support this claim.[2391] A UNAIDS Kuwait report stated that in 1999, four Kuwaiti prisoners out of 764 tested prisoners were found to be HIV-positive, and that in 2000, no prisoners out of 1503 prisoners tested were found to be HIV-positive.[2392] Based on the existence of HIV-positive prisoners, the account included in CNS' database seems possible.
March 9, 2000	Dr. Larry C. Ford, suspected of having orchestrated the attempted murder of his business partner, committed suicide.[2393] When police searched Ford's home, they found 266 bottles and vials of pathogens, including the causative agents of salmonella, cholera, botulism, and typhoid.[2394] Automatic weapons, explosives, and assassination paraphernalia – including the toxin ricin, and a blowgun and dart – were also found.[2395] The *New York Times* article on the incident presented a number of ultimately vague links between Ford and Apartheid South Africa's biological weapons program that had been uncovered; this included allegations that Ford had smuggled pathogens to South Africa.[2396]
February 25, 2000	The CNS database holds a case dated February 25, 2000, where a 17-year old student allegedly stabbed 37 classmates and a supervisor with a pin allegedly infected with HIV.[2397] As with the Kuwaiti case listed above, verifying that the case has occurred as described was not possible.
2000	Three sealed vials reportedly containing samples of coxsackie virus were found on board a passenger aircraft at the Sydney International Airport (Australia).[2398]

2386 Charles Choi, "Lab theft conviction: Former Cornell researcher found guilty of stealing valuable enzymes," *The Scientist*, December 17, 2002, http://www.the-scientist.com/?articles.view/articleNo/21813/title/Lab-theft-conviction/. Accessed September 7, 2015.
2387 Ibid.
2388 Ibid.
2389 Ibid.
2390 Jason Pate, Gary Ackerman, Kimberly McCloud, "2000 WMD Terrorism Chronology: Incidents Involving Sub-National Actors and Chemical, Biological, Radiological, or Nuclear Materials," *James Martin Center for Nonproliferation Studies (CNS)*, August 13, 2001, http://cns.miis.edu/reports/cbrn2k.htm. Accessed June 30, 2015.
2391 Ibid.
2392 Kuwait, "Epidemiological Fact Sheets on HIV/AIDS and Sexually Transmitted Infections," UNAIDS, 2004, p.2, http://data.unaids.org/publications/fact-sheets01/kuwait_en.pdf. Accessed June 30, 2015.
2393 Jo Thomas, "California Doctor's Suicide Leaves Many Troubling Mysteries Unsolved," *The New York Times*, November 3, 2002, p. 1, http://www.nytimes.com/2002/11/03/us/california-doctor-s-suicide-leaves-many-troubling-mysteries-unsolved.html?pagewanted=1. Accessed June 30, 2015.
2394 Ibid.
2395 Ibid.
2396 Ibid.
2397 Jason Pate, Gary Ackerman, Kimberly McCloud, "2000 WMD Terrorism Chronology: Incidents Involving Sub-National Actors and Chemical, Biological, Radiological, or Nuclear Materials."
2398 Hamid Mohtadi, Antu Murshid, "A Global Chronology of Incidents of Chemical, Biological, Radioactive and Nuclear Attacks: 1950-2005," July 7, 2006, http://www.ncfpd.umn.edu/Ncfpd/assets/File/pdf/GlobalChron.pdf. Accessed June 30, 2015.

Table 16.2. Biocrimes, 1990 to 2015	
December 1999 to January 2000	Two former postdocs from the Harvard Medical School signed an agreement in 2006 stating that they had stolen research data and materials, including cell lines and genetic material.[2399] The theft reportedly occurred over a five day period during the academic winter break, between 1999 and 2000.[2400]
August 1999	Medical waste was deliberately left in several locations in Norwalk and in Stamford (US).[2401] Two of the six containers sported swastikas and referenced a white supremacist charged with shooting five people at a Jewish community center.[2402,2403]
June 28, 1999	A burglar stole a physician's bag containing a vial with a sample of *Mycobacterium tuberculosis*.[2404,2405] The physician was planning to give the vial to a colleague at a medical conference (according to the media report of this incident, this was not illegal at the time).[2406] Police believed that the burglar did not know the bag contained the vial when the burglar stole the bag from the physician's hotel room.[2407]
Charged 1998	Larry Wayne Harris and William Job Leavitt Jr. were arrested by the FBI on charges of developing and stockpiling a biological agent and conspiring to use it as a weapon.[2408] According to *CNN* reporting, Leavitt's defense lawyer argued that the FBI had seized a substance his client had meant to test and market as an anthrax vaccine.[2409] The substance seized indeed turned out to be a veterinary vaccine strain of *B. anthracis*, and Harris was only charged with violation of his probation in relation to a prior incident.[2410] Harris, an individual associated with several white supremacist groups, had previously been arrested in 1995 for having forged documents to place an order for *Y. pestis* from a US laboratory.[2411] The order was placed with the American Type Culture Collection in Rockville, Maryland in the name of a fictitious "Small Animal Microbiology Laboratory," whose address was in reality Harris's home address.[2412] A sales representative who talked with Harris grew suspicious and called the Centers for Disease Control and Prevention to raise the alarm.[2413] Harris's home was then raided on May 12, 1995, and he was convicted of fraud on April 22, 1997.[2414] Harris' motivations during each instance is not known.

2399 Laurence H. M. Holland, "Couple Admits Cell Line Theft," *The Harvard Crimson*, April 17, 2006, http://www.thecrimson.com/article/2006/4/17/couple-admits-cell-line-theft-in/. Accessed September 10, 2015.
2400 Ibid.
2401 Ibid, p. 37.
2402 "Hate Waste: Task force formed after second container of medical waste found," *The Nevada Daily Mail*, August 20, 1999, 2A, retrieved at: https://news.google.com/newspapers?nid=1908&dat=19990820&id=rDEjAAAAIBAJ&sjid=0NkEAAAAIBAJ&pg=3962,3784318&hl=en. Accessed June 30, 2015.
2403 Rochelle Rosen, "Medical waste found in lot, Swastika drawn on container at Congregation Beth El school," *The Hour*, August 20, 1999, A1, retrieved at: https://news.google.com/newspapers?nid=1916&dat=19990820&id=uR9JAAAAIBAJ&sjid=SgYNAAAAIBAJ&pg=3253,2460359&hl=en. Accessed June 30, 2015.
2404 Hamid Mohtadi, Antu Murshid, "A Global Chronology of Incidents of Chemical, Biological, Radioactive and Nuclear Attacks: 1950-2005," p. 36.
2405 Associated Press (AP), "San Francisco police seeking TB vial stolen from researcher," *Deseret News*, June 29, 1999, http://www.deseretnews.com/article/704877/San-Francisco-police-seeking-TB-vial-stolen-from-researcher.html?pg=all. Accessed June 30, 2015.
2406 Ibid.
2407 Ibid.
2408 "2 charged with making biological weapons," *CNN*, February 19, 1998. http://www.cnn.com/US/9802/19/fbi.arrest.pm/#2. Accessed June 30, 2015.
2409 Ibid.
2410 Lauren Harisson, Jacqueline E. Miller, "Larry Wayne Harris," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 383-384.
2411 AAAS, AAU APLU, FBI, Bridging Science and Security for Biological Research: Personnel Security Programs, p. 37.
2412 Lauren Harisson, Jacqueline E. Miller, "Larry Wayne Harris," p. 383.
2413 Ibid.
2414 Ibid.

Table 16.2. Biocrimes, 1990 to 2015	
August 26, 1997	The New Zealand Ministry of Agriculture and Forestry announced on August 26, 1997 that rabbit haemorrhagic disease had been detected in New Zealand.[2415] As publicly suspected by the authorities, farmers admitted to having introduced the disease for use as a bio-control tool; its use had been considered but rejected by the New Zealand Ministry of Agriculture and Forestry.[2416]
October 1996	Laboratory technician Diane Thompson deliberately infected twelve co-workers at the St. Paul Medical Center hospital in Dallas, Texas (US) with *Shigella dysenteriae* type 2.[2417] She did so by sending out an anonymous email inviting colleagues to eat pastries she had covertly contaminated and left in the break room.[2418] Diane had ready access to a hospital laboratory holding the *Shigella dysenteriae* type 2 strain.[2419] Diane had previously contaminated her boyfriend in 1995, also using tainted food, and had then sabotaged his hospitalization records to prevent a correct diagnosis.[2420]
May 1996	Michael Just attempted to extort British dairies by threatening to contaminate their milk with *Yersinia enterocolitica*.[2421] He had obtained the pathogen by ordering it from a catalogue supply house.[2422] To prove that he was not bluffing, he included test tubes containing cultures of the pathogen in one blackmail package.[2423] The companies transferred money to a bank account; Just was arrested attempting to withdraw the funds.[2424]
August 1994	Dr. Richard J. Schmidt, a Louisiana gastroenterologist, deliberately infected a former lover with HIV and hepatitis using a contaminated hypodermic syringe.[2425] He subsequently misled health care professionals by telling them he had tested the victim for HIV with negative results.[2426]
February 6, 1992	Brian T. Stewart was found guilty of having deliberately infected his 11-month old son with HIV in an attempt to kill the boy.[2427] Stewart wanted to avoid paying child support.[2428] Stewart worked as a phlebotomist, a position which gave him access to the blood of HIV-positive patients.[2429] According to the prosecution, Stewart used this route to obtain contaminated blood, and then infected his son on February 6, 1992.[2430]
June 1992	"Iwan E." (court identifier for a Dutch man) injected his lover with HIV-contaminated blood drawn from an HIV-positive friend in June 1992.[2431] His lover had just broken up with him; the defense argued this was in a self-defense response to a knife threat made by the woman.[2432]

[2415] W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 42;
[2416] Ibid, p. 42-43.
[2417] W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 43-45;
Shellie A. Kolavic, Akiko Kimura, Shauna L. Simons, Laurence Slutsker, Suzanne Barth, Charles E. Haley, "An Outbreak of Shigella dysenteriae Type 2 Among Laboratory Workers Due to Intentional Food Contamination," *Biological Weapons: Limiting the Threat*, ed. Joshua Lederberg (Cambridge: The MIT Press, 1999), p. 186-192.
[2418] Raymond A. Zilinskas, "Diane Thompson: A Case Study," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 238.
[2419] Ibid.
[2420] Ibid.
[2421] W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*.
[2422] Ibid.
[2423] Ibid.
[2424] Ibid.
[2425] AAAS, AAU APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*.
[2426] Ibid.
[2427] W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*.
[2428] Ibid.
[2429] Ibid.
[2430] Ibid.
[2431] Ibid.
[2432] Ibid.

Table 16.2. Biocrimes, 1990 to 2015	
July 1990	Graham Farlow, inmate at a prison in New South Wales (Australia), infected a prison warder with his HIV-contaminated blood through assault with a syringe.[2433,2434]
1990?	According to reports by physicians published in early 1991, a French woman attempted suicide by injection of HIV-contaminated blood drawn from a friend who had AIDS.[2435]
November 1984	Kevin T. Birch and James B. Cahoon were convicted of having obtained pathogens under false pretenses.[2436] FBI simply stated that this was for "personal gain;" the media suggested they had intended to kill a race horse.[2437] The two Canadian men pretended to be from "ICM Science Ltd." to order freeze-dried *Clostridium tetani* from the American Type Culture Collection.[2438] When ICM Science Ltd. received a copy of the shipping invoice, they discovered the subterfuge and contacted the police.[2439] The two men attempted to effectuate a second shipment, this time for *Clostridium botulinum* as well as for *Clostridium tetani*, and were arrested.[2440]

16.8 Terrorist and Extremist Events Tied to Biological Warfare

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)		
August 28, 2014	Islamic State of Iraq and the Levant (ISIL, ISIS, Daesh)	*Foreign Policy* journalists report on the obtained contents of one alleged ISIL member's laptop.[2441] It held over 35,000 files dedicated to Jihad, a few of which discussed BW.[2442]
2013	Communist Party of the Philippines/ New People's Army (CPP/NPA)	Philippines military claims that NPA used feces to spike explosive devices to cause sepsis, in what appears to be a modern take on the Viet Cong punji stick technique.[2443] The NPA denies this.[2444]
May 2012	Revolutionary Armed Forces of Colombia (FARC)	A defused FARC gas cylinder bomb reportedly had feces mixed with shrapnel in order to cause sepsis upon injury.[2445]

[2433] Ibid.
[2434] Philip D. Jones, "HIV transmission by stabbing despite zidovudine prophylaxis." *Lancet* (Letter) 338 October 5, 1991.
[2435] W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*.
[2436] Ibid.
[2437] Ibid.
[2438] Ibid.
[2439] Ibid.
[2440] Ibid.
[2441] Harald Doornbos, Jenan Moussa, "Found: The Islamic State's Terror Laptop of Doom," *Foreign Policy*, August 28, 2014, http://foreignpolicy.com/2014/08/28/found-the-islamic-states-terror-laptop-of-doom/. Accessed June 30, 2015.
[2442] Ibid.
[2443] "Philippine Army finds human feces, snake venom in wounded soldiers' wounds," *Nindanao Examiner*, September 4, 2013, http://nindanaoexaminer.com/philippine-army-finds-human-feces-snake-venom-in-wounded-soldiers-wounds/. Accessed June 30, 2015.
[2444] Ibid.
[2445] "Army destroys minefield in southwest Colombia," *Colombia Reports*, May 17, 2012, http://colombiareports.com/minefield-and-explosives-found-in-southwest-colombia/. Accessed August 11, 2015.

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)		
2010	"Indian Mujahedeen (Assam)"	A 2010 email claiming to be from the "Indian Mujahedeen (Assam)" group threatens biological warfare against India unless its demands are met.[2446] However, no evidence exists that this group had or has a BW capability.
After 2009, up to 2011	Al Qaeda (AQ Central)	Senior AQ member Abu-Salih al Somali authors "Terror Franchise: The Unstoppable Assassin, TECHS Vital role for its success" sometime after 2009.[2447] The document ends with a detailed list of military topics about which the author is requesting the "techs" to research and share instruction manuals and videos. BW topics figure prominently on this list, and are marked as "immediately needed."[2448] The document is captured in the 2011 raid that killed Bin Laden.[2449]
2009	Al Qaida in the Islamic Maghreb (AQIM)	Highly contested news reports of a BW training camp accident.[2450][2451][2452]

[2446] "Extremists Warn of Biological Strike in India," *Nuclear Threat Initiative Global Security Newswire*, October 4, 2010, http://www.nti.org/gsn/article/extremists-warn-of-biological-strike-in-india/. Accessed June 30, 2015.

[2447] David Francis, "Al Qaeda's Blueprint For How To Start a Homegrown Terror Franchise," *Foreign Policy*, May 20, 2015, http://foreignpolicy.com/2015/05/20/al-qaedas-blueprint-for-how-to-start-a-homegrown-terror-franchise/. Accessed June 30, 2015.

[2448] Office of the Director of National Intelligence, Bin Laden's Bookshelf," http://www.dni.gov/index.php/resources/bin-laden-bookshelf?start=1. Retrieved under the "Now Declassified Material" folder: Abu-Salih Al Somali, "Terror Franchise: The Unstoppable Assassin, TECHS Vital role for its success," p. 2, 5, 10, http://www.dni.gov/files/documents/ubl/english/Terror%20Franchise.pdf. Accessed June 30, 2015.

[2449] Ibid.

[2450] For a critical review of these accounts, see: Rene Pita, Rohan Gunaratna, Philip Henika, "Al Qaeda in the Islamic Maghreb (AQIM) and the Alleged Production of the Etiological Agent of Plague," *ISA Newsletter* 131 (April 2009): p. 1, 21-22, http://www.asanltr.com/newsletter/09-2/articles/092a.pdf. Accessed July 17, 2015.

[2451] For the accounts themselves, see:
Eli Lake, "Al Qaeda bungles arms experiment," *The Washington Times*, January 19, 2009, http://www.washingtontimes.com/news/2009/jan/19/al-qaeda-bungles-arms-experiment/. Accessed July 14, 2015. And:.

[2452] Olivier Guitta, "Al-Qaeda in the Islamic Maghreb: A Threat for the West," *Defence Against Terrorism Review* 3, no. 1 (Spring 2010): p. 57-58, http://www.coedat.nato.int/publication/datr/volume5/03-Al-Qaeda_in_the_Islamic_Maghreb_A_Threat_for_the_West.pdf. Accessed July 14, 2015.

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)

July 17, 2008	[Aafia Siddiqui alleged case]	The FBI's complaint filing against Aafia Siddiqui during her trial stated that at the time of Aafia Siddiqui's arrest on July 17, 2008, Afghanistan National Police found "numerous chemical substances in gel and liquid form that were sealed in glass bottles and glass jars," as well as "numerous documents describing the creation of explosives, chemical weapons, and other weapons involving biological material and radiological agents," "documents detailing United States military assets" personal papers including "descriptions of various landmarks in the United States, including in New York City," and "handwritten notes that referred to a '' 'mass casualty attack' " and that listed various locations in the United States, including Plum Island, the Empire State Building, the Statue of Liberty, Wall Street, and the Brooklyn Bridge."[2453] The government's sentencing submission for the case also holds that her "thumb drive contained documents [...] including: [...] discussions of the construction of chemical and biological weapons."[2454] The prosecution argued that Aafia Siddiqui's "conduct was the very definition of a federal crime of terrorism."[2455] The media reported to the effect that she was a "suspected al-Qaeda operative;" Saddiqui and her family deny this allegation, and her trial did not involve an assessment of this accusation.[2456,2457,2458,2459,2460] Since then, the Taliban, the Tehrik-i-Taliban Pakistan, Al Qaeda, and most recently ISIL have offered (some on multiple occasions) to trade Saddiqui against hostages.[2461,2462,2463,2464,2465,2466]

[2453] Plum Island is the site of the Plum Island Animal Disease Center, although the prosecution did not elaborate on the alleged targets.
United States District Court Southern District of New York, United States of America v. Aafia Siddiqui (defendant). "Sealed Complaint: Violations of 18 U.S.C. §§ 111, 1114, p. 1-3, http://www.justice.gov/archive/opa/pr/2008/August/siddiqui-aafia-complaint.pdf. Accessed June 30, 2015.

[2454] United States District Court Southern District of New York, United States of America v. Aafia Siddiqui (defendant). "Government's Sentencing Submission." Attorney for the United States of America: Preet Bharara, United States Attorney for the Southern District of New York, Assistant United States Attorneys – of Counsel, Christopher L. LaVigne, David M. Rody, Jenna M. Dabbs, Case 1:08-cr-00826-RMB, Document 250, Filed August 29, 2010. http://web.archive.org/web/20120314163620/http://www.nefafoundation.org/miscellaneous/US_v_Siddiqui_usgsentmemo.pdf . Accessed June 30, 2015.

[2455] Ibid.

[2456] "Dr. Aafia to boycott trial," *The Nation*, November 21, 2009, http://nation.com.pk/Politics/21-Nov-2009/Dr-Aafia-to-boycott-trial. Accessed June 30, 2015.

[2457] Benjamin Weiser, "Indictment Hints of Plan to Attack Landmarks," *The New York Times*, September 2, 2008, http://www.nytimes.com/2008/09/03/nyregion/03indict.html?_r=1&.

[2458] Petra Bartosiewicz, "Al-Qaeda Woman? Putting Aafia Siddiqui on Trial," *Time*, January 18, 2010, http://content.time.com/time/nation/article/0,8599,1954598,00.html.

[2459] Juliane von Mittelstaedt, "America's Most Wanted: 'The Most Dangerous Woman in the World,' *Spiegel Online*, November 27, 2008, http://www.spiegel.de/international/world/america-s-most-wanted-the-most-dangerous-woman-in-the-world-a-593195-druck.html.

[2460] "Federal jury convicts Pakistani woman of attempted murder of US personnel," *Jurist*, February 4, 2010, http://jurist.org/paperchase/2010/02/federal-jury-convicts-pakistani-woman.php.

[2461] Mushtaq Yusufzai, "Taliban to execute US soldier if Aafia not released," *The News*, February 5, 2010, http://www.webcitation.org/query?url=http%3A%2F%2Fwww.thenews.com.pk%2Ftop_story_detail.asp%3FId%3D27072.

[2462] Bill Roggio, "Zawahiri claims al Qaeda is holding US citizen hostage," *Long War Journal – Threat Matrix*, December 1, 2011, https://web.archive.org/web/20150103043251/http://www.longwarjournal.org/threat-matrix/archives/2011/12/zawahiri_claims_al_qaeda_holdi.php.

[2463] "Taliban confirm they have Swiss hostages," *Agence France Presse*, July 29, 2011, retrieved at *The Express Tribune*: http://tribune.com.pk/story/220022/tehrik-i-taliban-say-they-have-swiss-hostages/.

[2464] Nima Elbagir, Ingrid Formanek, "Malian troops take key town; humanitarian crisis grows," *CNN*, January 21, 2013, http://www.cnn.com/2013/01/21/world/africa/mali-unrest/.

[2465] Jan Lopatka, ed. Alison Williams, "Video of kidnapped Czechs demands release of jailed Pakistani," *Reuters*, June 26, 2013, http://www.reuters.com/article/2013/06/26/us-pakistan-czech-kidnapping-idUSBRE95P0XJ20130626.

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)

2008	East Turkistan Islamic Movement	The Chinese government alleges that Emeti Yakuf, an alleged terrorist connected to the East Turkistan Islamic Movement, threatened to use biological and chemical weapons to disrupt the 2008 Olympics held in China, and that he trained group members on making poisons.[2467] This individual was reportedly killed in a 2012 US drone strike in Pakistan.[2468]
June 27, 2006	Al-Aqsa Martyrs Brigade	The group issues a statement claiming that they possess chemical and biological weapons, in an attempt to deter Israeli military action.[2469] This claim is regarded as spurious.[2470]
April 4, 2003	Ansar al-Islam (AAI)	MSNBC reporters state that their initial field tests for botulinum and ricin toxins came up positive at a site in Iraq used by the group, but that no *B. anthracis* was detected; then-Secretary of State Colin Powell had previously said the camp held a poison laboratory.[2471] However, in retrospect, the site does not appear to have produced toxins. The site is not mentioned in the report of the Iraq Survey Group.[2472]
August 2003	Jemaah Islamiyah	Arrest of Riduan Isamuddin, the director of operations for Jemaah Islamiyah who organized for Yazid Sufaat's transfer to AQ.[2473,2474]
June 2002	Revolutionary Armed Forces of Colombia (FARC)	A defused FARC gas cylinder bomb reportedly had feces mixed with shrapnel in order to cause sepsis upon injury.[2475]
December 2001	Al Qaeda (AQ Central); Jemaah Islamiyah	Rauf Ahmed is detained in Pakistan, and Yazid Sufaat is arrested in Malaysia.[2476,2477] Pakistan subsequently cuts off FBI access to Rauf Ahmed in 2003; the latter is now free.[2478]

[2466] James Fielding, Marco Giannangeli, "British Aid Worker Executed By Taliban," *Daily Express*, October 10, 2013, http://web.archive.org/web/20101015002351/http://www.dailyexpress.co.uk/posts/view/204533/British-aid-worker-executed-by-Taliban.

[2467] "'Eastern Turkistan' terrorists identified," *China Daily*, October 21, 2008, http://www.chinadaily.com.cn/china/2008-10/21/content_7126503.htm.

[2468] Declan Walsh, Eric Schmitt, "Militant Leader Believed Dead in Pakistan Drone Strike," *The New York Times*, August 24, 2012, http://www.nytimes.com/2012/08/25/world/asia/us-drone-strikes-kill-18-in-pakistan.html?_r=1.

[2469] "Al-Aqsa Martyrs Brigade in Palestine Claims to Have Developed Chemical and Biological Weapons and Threatens Their Use in Israel," *SITE Monitoring Service Enterprise*, June 27, 2006, https://ent.siteintelgroup.com/Jihadist-News/6-27-06-al-aqsa-martyrs-in-palestine-creates-wmd.html.

[2470] Michael Moodie, Markus Binder, "Jihadists and Chemical Weapons," *Jihadists and Weapons of Mass Destruction*, eds. Gary Ackerman, Jeremy Tamsett (Boca Raton: CRC Press, 2009), p. 143.

[2471] Preston Mendenhall, "Positive test for terror toxins in Iraq," *MSNBC.com*, April 4, 2003, http://www.nbcnews.com/id/3070394/ns/world_news-t/positive-test-terror-toxins-iraq/#.VXdWckbrJ-A.

[2472] Milton Leitenberg, *Assessing the Biological Weapons and Bioterrorism Threat*, Strategic Studies Institute monograph, December 2005, p. 26-27, http://www.strategicstudiesinstitute.army.mil/pdffiles/pub639.pdf.

[2473] Joel Roberts, "Thailand PM: Hambali Was Plotting," *CBS News*, August 17, 2003, http://www.cbsnews.com/news/thailand-pm-hambali-was-plotting/.

[2474] Rolf Mowatt-Larssen, "Al Qaeda Weapons of Mass Destruction Threat: Hype or Reality?" Paper, Harvard Kennedy School Belfer Center for Science and International Affairs, January 2010, p. 28, http://belfercenter.ksg.harvard.edu/publication/19852/al_qaeda_weapons_of_mass_destruction_threat.html.

[2475] Mariano C. Bartolome, Maria Jose Espona, "Chemical and Biological Terrorism in Latin America: The Revolutionary Armed Forces of Colombia," *The ASA Newsletter* 03-5, no. 98 (October 31, 2003), http://www.asanltr.com/newsletter/03-5/articles/035c.htm.

[2476] Joby Warrick, "Suspect and A Setback in Al-Qaeda Anthrax Case," *The Washington Post*, October 31, 2006, http://www.washingtonpost.com/wp-dyn/content/article/2006/10/30/AR2006103001250.html.

[2477] Maria Ressa, "Reports: Al Qaeda operative sought anthrax," *CNN*, October 10, 2003, http://edition.cnn.com/2003/WORLD/asiapcf/southeast/10/10/alqaeda.anthrax/.

[2478] Joby Warrick, "Suspect and a Setback in Al-Qaeda Anthrax Case."

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)

2001	Jemaah Islamiyah	Yazid Sufaat flees Afghanistan for Bogor, Indonesia, to escape from the October 2001 US intervention.[2479] He reportedly seeks to set up a new BW program in-country upon arrival, but fails to recruit a microbiologist at an Indonesian institute.[2480,2481,2482]
September and October 2001	[Amerithrax case]	"At least five envelopes containing significant quantities of *Bacillus anthracis*" were mailed to US targets.[2483] The attacks killed five and sickened seventeen other individuals.[2484] FBI concluded that Bruce E. Ivins, a researcher at USAMRIID (US) had sent the letters.[2485]
1999-2001	Al Qaeda (AQ Central); Jemaah Islamiyah	Zawahiri launches a BW program in 1999, and hires Rauf Ahmed.[2486,2487] Ahmed establishes a covert laboratory in Afghanistan.[2488] By 2000, Zawahiri recruits Yazid Sufaat.[2489] US outing of the Taliban disrupts the plan and the laboratory is discovered.[2490,2491]
1998 to May 2000	"Palestinian Group"	A Palestinian group (unknown) was reportedly caught in a counterfeiting scheme whereby expired eggs contaminated with salmonella were stamped with counterfeit stamps and sold.[2492] Israeli news reporting on their capture in May 2000 implied that this was deliberately done to sicken Israelis.[2493]
February 1999	Chechen group under Salman Raduyev	One Russian newspaper claimed that Salman Raduyev, a prominent Chechen leader, had threatened to steal biological weapons from ex-Soviet biological warfare laboratories unless the government released two captured women.[2494] This report could not be verified.[2495]
June 1998	"Republic of Texas"	Two members of the group sent emails threatening to use biological agents against federal officials; no biological agents were uncovered at the time of their arrest.[2496]

[2479] Judith Miller, "U.S. Has New Concerns About Anthrax Readiness," *The New York Times*, December 28, 2003, http://www.nytimes.com/2003/12/28/us/us-has-new-concerns-about-anthrax-readiness.html.
[2480] Ibid.
[2481] Maria Ressa, "Reports: Al Qaeda operative sought anthrax;"
[2482] René Pita, Rohan Gunaratna, "Revisiting Al-Qa'ida's Anthrax Program,"
[2483] The United States Department of Justice, "Amerithrax Investigative Summary, Released Pursuant to the Freedom of Information Act," February 19, 2010, p. 1, http://www.justice.gov/archive/amerithrax/docs/amx-investigative-summary.pdf.
[2484] Ibid.
[2485] Ibid.
[2486] Alan Cullison, "Inside Al-Qaeda's Hard Drive," *The Atlantic*, September 2004, http://www.theatlantic.com/magazine/archive/2004/09/inside-al-qaeda-s-hard-drive/303428/.
[2487] Rolf Mowatt-Larssen, "Al Qaeda Weapons of Mass Destruction Threat: Hype or Reality?"
[2488] Ibid.
[2489] René Pita, Rohan Gunaratna, "Revisiting Al-Qa'ida's Anthrax Program," *CTC Sentinel* Vol. 2 Issue 5, May 2009, https://www.ctc.usma.edu/posts/revisiting-al-qaida%E2%80%99s-anthrax-program.
[2490] Ibid.
[2491] Rolf Mowatt-Larssen, "How to Get Terrorists to Talk," *The National Interest*, February 18, 2015, p.2, http://nationalinterest.org/feature/how-get-terrorists-talk-12270?page=2.
[2492] Jason Pate, Gavin Cameron, "Covert Biological Weapons Attacks against Agricultural Targets: Assessing the Impact against U.S. Agriculture," BCSIA Discussion Paper 2001-9, ESDP Discussion Paper ESDP-2001-05, John F. Kennedy School of Government, Harvard University, August 2001, p.8, http://belfercenter.ksg.harvard.edu/files/covert_biological_weapons_attacks_against_agricultural_targets.pdf.
[2493] Ibid.
[2494] W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, Working Paper (Washington: Center for Counterproliferation Research, National Defense University, February 2001 Revision), p. 107.
[2495] Ibid.
For instance, the Russian think-tank PIR Center does not include this incident in their list of North Caucasus CBRN threat events. PIR Center, "WMD Terrorism Originated in North Caucasus: Again on the Agenda?" *PIR Center Report*, April 26, 2013, http://www.pircenter.org/en/articles/1312-wmd-terrorism-originated-in-north-caucasus-again-on-the-agenda.
[2496] W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 108-109, p. 186.

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)

April 1998	Palestinian Islamic Jihad	A Jordanian newspaper cites a leading figure in the organization as having discussed the possibility of using BW.[2497] This remains unconfirmed.
March 6, 1998	National Liberation Army (ELN)	The ELN detonate an explosive device reportedly spiked with fecal matter to cause sepsis upon injury.[2498]
1997	Counter Holocaust Lobbyists of Hillel	Agar and *B. cereus* in a petri dish apparently labelled "*anthracs" [SIC]* and "*Yersinia*" was sent to a Jewish organization in Washington.[2499] Whether this was an anthrax hoax or the group thought the package contained *B. anthracis* is not known; the package contained a hate letter that further misidentified the petri dish as containing a "chemical warfare" agent.[2500]
1996	"Justice Department" [animal rights radical group]	A group calling itself the "Justice Department" mails razors to fur retailers in Canada in 1996 which they claim are covered with HIV-infected blood; whether they really did so is not known.[2501]
March 15, 1995	Aum Shinrikyo	The group ineffectually attempts to disperse botulinum toxin from three sprayer-suitcases in the Kasumigaseki metro station (Japan).[2502]
November 4, 1994	Aum Shinrikyo	The group fails in an assassination attempt involving botulinum toxin mixed with juice.[2503]
1993	Animal Liberation Front (ALF) [animal rights radical group]	A spokesman for the Animal Liberation Front (ALF) claims that bombs planted in the UK by members of the collective had been purposefully tainted with HIV, but authorities dismiss this account.[2504]
November 18, 1993	Aum Shinrikyo	The group disperses 20 liters of botulinum toxin slurry from a car sprayer in a failed assassination attempt.[2505]
1993	Aum Shinrikyo	Following failed attacks with the liquid product, the group sets up a (crude) dry production line for *B. anthracis*.[2506]
July-August 1993	Aum Shinrikyo	The group produces some 10 to 20 tons of slurry containing *B. anthracis* (perhaps not pathogenic), which are then ineffectually released from spray trucks in some 10 to 20 attacks.[2507]

2497 Ibid.
2498 Mariano C. Bartolome, Maria Jose Espona, "Chemical and Biological Terrorism in Latin America: The Revolutionary Armed Forces of Colombia."
2499 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 110-111; The B'nai B'rith International Jewish Monthly, Volume 111, (1996), p. 67, https://books.google.com/books?id=V--3AAAAIAAJ&q=anthracis+Yersinia+Counter+Holocaust+Lobbyists+of+Hillel&dq=anthracis+Yersinia+Counter+Holocaust+Lobbyists+of+Hillel&hl=en&sa=X&ved=0CC8Q6AEwA2oVChMI98TMwLKIxgIVOEaMCh0gNAC0.
2500 W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 111.
2501 Ibid.
2502 Richard Danzig, Marc Sageman, Terrance Leighton, Lloyd Hough, Hidemi Yuki, Rui Kotani, Zachary M. Hosford, "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition." Center for a New American Security, December 2012, p. 21, http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf.
2503 Ibid.
2504 W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 76.
2505 Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition,"
2506 Ibid.
2507 Ibid.

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)		
May-June 1993	Aum Shinrikyo	The group produces roughly 20 tons of slurry containing *B. anthracis* (perhaps not pathogenic), and ineffectually sprays the product from the roof of one of its facilities.[2508]
1992	Aum Shinrikyo	The group sets up a (crude) liquid production line for *B. anthracis*.[2509]
March-July 1990	Aum Shinrikyo	The group produces several hundred tons of slurry as part of their botulinum toxin production program.[2510] They disseminate this material in 20 to 40 different attempted attacks in this time period, all without success.[2511]
Spring 1990	Aum Shinrikyo	Seiichi Endo, the leader of the group's BW program, harvests *C. botulinum* from soil in Japan.[2512]
September 1984	Rajneeshees	*S. typhimurium* is used to contaminate at least 10 restaurant salad bars in The Dalles, Oregon (US), causing at least 751 people to fall ill.[2513,2514,2515]
August 29, 1984	Rajneeshees	Two Wasco County commissioners were given water deliberately tainted with *S. typhimurium* by Rajneeshees; both fell ill.[2516]
Early 1984	Rajneeshees	Reports, based on admissions made by Rajneesh members, of other cult BW attacks prior to August 1984.[2517] These are unconfirmed because none of the attacks were successful and because there may have been a desire to exaggerate wrongdoings by one of the chief organizers (Puja), who was hated.[2518]
October 14, 1981	Dark Harvest [eco-radical group]	In an apparent follow-on to the October 10, 1981 incident described below, British police received an anonymous tip that led them to a metal box allegedly containing *B. anthracis*.[2519] However, unlike in the October 10 incident, the soil did not contain *B. anthracis*.[2520]

2508 Ibid.
2509 Ibid.
2510 Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," *Center for a New American Security*, December 2012, p. 20, http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf>.
2511 Ibid.
2512 Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," *Center for a New American Security*, December 2012, p. 18-20, http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf.
2513 Thomas J. et. al (1997), "A Large Community Outbreak of Salmonellosis Caused by Intentional Contamination of Restaurant Salad Bars," *Journal of the American Medical Association* 278, no. 5, http://www.cdc.gov/phlp/docs/forensic_epidemiology/Additional%20Materials/Articles/Torok%20et%20al.pdf.
2514 W. Seth Carus, "The Rajneeshees (1984)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan Tucker (Cambridge: The MIT Press, 2001), p. 115;
2515 W. Seth Carus, "Rajneeshees," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 383-384.
2516 W. Seth Carus, "Rajneeshees," p. 534.
2517 Ibid, p. 534-535.
2518 W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 534.
2519 Ibid.
2520 Ibid.

Table 16.3. Chronology of Terrorist and Extremist Events Tied to Biological Warfare (BW)		
October 10, 1981	Dark Harvest [eco-radical group]	The eco-radical group "Dark Harvest" took *B. anthracis*-contaminated soil from Gruinard Island (a then-contaminated British military WWII site used to test *B. anthracis* bombs) and spread it on the grounds of Porton Down in 1981 (Britain's main biodefense and chemical warfare defense establishment, and previously the center orchestrating Britain's biological weapons program).[2521] The soil did contain *B. anthracis*.[2522]
1980s	Tamil "militants"	A single unconfirmed account of Tamil "militants" threatening biological warfare.[2523]
October 1980	Red Army Faction	The German-based, now-defunct Red Army Faction (RAF) reportedly maintained a botulinum toxin laboratory in Paris, France until it was uncovered in October 1980.[2524] A recent review of this case has cast doubt on parts of the underlying story, however, and German authorities apparently remain convinced that "no evidence whatsoever [exists] that members of the 'RAF' had planned or prepared an attack using biological agents."[2525,2526]
February 1975	POLISARIO: Basque Fatherland and Liberty (ETA)	One unconfirmed report of a February 1975 offer by a group called POLISARIO to coordinate poisoning of water supplies.[2527] Even if POLISARIO did make such a threatening offer, no evidence exists that POLISARIO sought a BW capability.[2528,2529]
January 18, 1972	R.I.S.E.	Arrest of two R.I.S.E. founders for having reportedly planned to contaminate Chicago's municipal water system with *Salmonella typhi* (causative agent of typhoid fever).[2530]

16.9 Designated Foreign Terrorist Organizations and Biological Weapons

Table 16.4. Currently Designated Foreign Terrorist Organizations and BW				
Abu Nidal Organization (ANO)	NO	NO		

2521 W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 58.
2522 Ibid.
2523 Ibid.
2524 W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 156-157.
2525 Ibid.
2526 The review in question is:
Terence Taylor, Tim Trevan, "The Red Army Faction (1980)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan B. Tucker (Cambridge: MIT Press, 2000).
2527 W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 121.
2528 POLISARO stands for "Frente Popular para la Liberacion de Saquia el-Hamra y Rio de Oro," and is a group that seeks to overthrow Moroccan control of Western Sahara and create an independent state for Sahrawi tribes based on Islamic culture.
2529 Gail H. Nelson, "POLISARIO," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 510-512.
2530 W. Seth Carus, "RISE: A Case Study," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 542.

Table 16.4. Currently Designated Foreign Terrorist Organizations and BW				
Abu Sayyaf Group (ASG)	NO	NO		A single news report that police captured publicly-available reading material on biological and chemical warfare during the capture of six suspected group members.[2531] Whether the group members were members of ASG or JI is unclear.
Aum Shinrikyo (AUM)	NO	**YES**	NO	Attempted production of BW agent; launched failed BW attacks. See the detailed entry below. Leadership and BW-program members captured.
Basque Fatherland and Liberty (ETA)	NO	NO		One unconfirmed report of a February 1975 offer by a group called Polisario to coordinate poisoning of water supplies.[2532]
Gama'a al-Islamiyya (Islamic Group) (IG)	NO	NO		
Hamas	NO	NO		Reported interest in chemical poisons.[2533]
Harakat ul-Mujahidin (HUM)	NO	NO		
Hizballah	NO	NO		
Kahane Chai (Kach)	NO	NO		
Kurdistan Workers Party (PKK) (Kongra-Gel)	NO	NO		A single unconfirmed Turkish newspaper report of Cobra poison smuggling for profit.[2534]
Liberation Tigers of Tamil Eelam (LTTE)	**Unconfirmed**	NO	NO	A single unconfirmed account of Tamil "militants" threatening biological warfare in the 1980s.[2535] Report of LTTE use of chlorine for chemical warfare.[2536] Group has been defeated.

[2531] Christian Enemark, Disease and Security: Natural plagues and biological weapons in East Asia (Abingdon: Routledge, 2007), p. 106.

[2532] W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, Working Paper (Washington: Center for Counterproliferation Research, National Defense University, February 2001 Revision), p. 121.

[2533] Ibid.

[2534] Ibid.

[2535] Ibid.

[2536] James J. F. Forest, Sammy Salama, "Jihadist Tactics and Targeting," *Jihadists and Weapons of Mass Destruction*, eds. Gary Ackerman, Jeremy Tamsett (Boca Raton: CRC Press, 2009), p. 80.

Table 16.4. Currently Designated Foreign Terrorist Organizations and BW				
National Liberation Army (ELN)	NO	**NO, albeit reported war use of biological material**	**Unknown if continuing war use of biological material**	Reports that ELN used feces to spike explosive devices to cause sepsis, in what appears to be a modern take on the Viet Cong punji stick technique.[2537]
Palestine Liberation Front (PLF)	NO	NO		
Palestinian Islamic Jihad (PIJ)	NO	NO		A single unconfirmed April 1998 Jordanian newspaper report citing a leading figure in the organization as having discussed the possibility of using BW.[2538]
Popular Front for the Liberation of Palestine (PFLF)	NO	NO		
PFLP-General Command (PFLP-GC)	NO	NO		
Revolutionary Armed Forces of Colombia (FARC)	NO	**NO, albeit reported war use of biological material**	**Continued war use of biological material**	Reports that FARC used feces to spike explosive devices to cause sepsis, in what appears to be a modern take on the Viet Cong punji stick technique.[2539,2540,2541]
Revolutionary Organization 17 November (17N)	NO	NO		
Revolutionary People's Liberation Party/Front (DHKP/C)	NO	NO		
Shining Path (SL)	NO	NO		
al-Qa'ida (AQ)	**YES**	**YES**	**YES**	Attempted production of BW agent, with unknown results. See detailed entry below. Efforts believed to be ongoing.

[2537] Mariano C. Bartolome, Maria Jose Espona, "Chemical and Biological Terrorism in Latin America: The Revolutionary Armed Forces of Colombia," *The ASA Newsletter* 03-5, no. 98 (October 31, 2003), http://www.asmltr.com/newsletter/03-5/articles/035c.htm.

[2538] W. Seth Carus, Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900, p. 109, 186.

[2539] Mariano C. Bartolome, Maria Jose Espona, "Chemical and Biological Terrorism in Latin America: The Revolutionary Armed Forces of Colombia".

[2540] Pablo Esteban Parra Gallego, "IEDs: A Major Threat for a Struggling Society," *The Journal of ERW and Mine Action* 13, no. 3 (Winter 2009), http://www.jmu.edu/cisr/journal/13.3/specialreport/gallego/gallego.shtml

[2541] "Army destroys minefield in southwest Colombia," *Colombia Reports*, May 17, 2012, http://colombiareports.com/minefield-and-explosives-found-in-southwest-colombia/.

Table 16.4. Currently Designated Foreign Terrorist Organizations and BW				
al-Qaida in the Islamic Maghreb (AQIM)	**YES** (by proxy with AQ)	**YES**	**YES**	By proxy with AQ (central): highly contested news reports of a BW training camp accident in 2009.[2542,2543,2544]
al-Qa'ida in the Arabian Peninsula (AQAP)	**YES** (by proxy with AQ)	**Unknown**		Possibly by proxy with AQ (central). No information formally ties this group with AQ's BW program. The group reportedly considered contaminating US food with ricin and cyanide, although no open source indications suggest the group selected this tactic for operationalization.[2545]
Islamic Movement of Uzbekistan (IMU)	NO	NO		
Real Irish Republican Army (RIRA)	NO	NO		
Jaish-e-Mohammed (JEM)	NO	NO		
Lashkar-e Tayyiba (LeT)	NO	NO		
Al-Aqsa Martyrs Brigade (AAMB)	**YES**	NO		The group claimed to possess chemical and biological weapons in 2006 in an attempt to deter Israeli military action.[2546] This claim is regarded as spurious.[2547]
Asbat al-Ansar (AAA)	NO	NO		
Communist Party of the Philippines/New People's Army (CPP/NPA)	NO	**NO, albeit reported war use of biological material**		Recent Philippines military claim that NPA used feces to spike explosive devices to cause sepsis; see FARC and ELN entries.[2548] The NPA denies this.

2542 For a critical review of these accounts, see: René Pita, Rohan Gunaratna, Philip Henika, "Al Qaeda in the Islamic Maghreb (AQIM) and the Alleged Production of the Etiological Agent of Plague," *ASA Newsletter* 131 (April 2009): p. 1, 21-22. http://www.asanltr.com/newsletter/09-2/articles/092a.pdf.

2543 For the accounts themselves, see:
Eli Lake, "Al Qaeda bungles arms experiment," *The Washington Times*, January 19, 2009, http://www.washingtontimes.com/news/2009/jan/19/al-qaeda-bungles-arms-experiment/

2544 Olivier Guitta, "Al-Qaeda in the Islamic Maghreb: A Threat for the West," *Defence Against Terrorism Review* 3, no. 1 (Spring 2010): p. 57-58, http://www.coedat.nato.int/publication/datr/volume5/03-Al-Qaeda_in_the_Islamic_Maghreb_A_Threat_for_the_West.pdf.

2545 Mike M. Ahlers, Brian Todd, "Al Qaeda group contemplated poisoning food in U.S., officials say," December 22, 2010, http://www.cnn.com/2010/US/12/21/al.qaeda.poison.plot/.

2546 Michael Moodie, Markus Binder, "Jihadists and Chemical Weapons," *Jihadists and Weapons of Mass Destruction*, eds. Gary Ackerman, Jeremy Tamsett (Boca Raton: CRC Press, 2009), p. 143.

2547 Ibid.

2548 "Philippine Army finds human feces, snake venom in wounded soldiers' wounds," *Mindanao Examiner*, September 4, 2013, http://mindanaoexaminer.com/philippine-army-finds-human-feces-snake-venom-in-wounded-soldiers-wounds/.

Table 16.4. Currently Designated Foreign Terrorist Organizations and BW				
Jemaah Islamiya (JI)	**YES** (By proxy with AQ)	**YES**	NO	Attempted production of BW, mostly as part of Al Qaeda's program, with unknown results. See detailed entry. Group membership, including leadership and individuals involved in the BW program, decimated.
Lashkar i Jhangvi (LJ)	NO	NO		Pakistani police reportedly uncovered chemical laboratories belonging to the group.[2549]
Ansar al-Islam (AAI)	NO	**Unsubstantiated reports of interest in toxins**	NO	Initial reports held that the group had a poison laboratory in Iraq that manufactured botulinum and ricin toxin.[2550] However, in retrospect, the site does not appear to have produced toxins. The site is not mentioned in the report of the Iraq Survey Group.[2551]
Continuity Irish Republican Army (CIRA)	NO	NO		
Libyan Islamic Fighting Group (LIFG)	NO	NO		

[2549] National Consortium for the Study of Terrorism and Responses to Terrorism (START), "Terrorist Organization Profiles: Lashkar-e-Jhangvei," http://www.start.umd.edu/tops/terrorist_organization_profile.asp?id=65.

[2550] Preston Mendenhall, "Positive test for terror toxins in Iraq," *MSNBC.com*, April 4, 2003, http://www.nbcnews.com/id/3070394/ns/world_news-t/positive-test-terror-toxins-iraq/#.VXdWckbrJ-A.

[2551] Milton Leitenberg, *Assessing the Biological Weapons and Bioterrorism Threat*, Strategic Studies Institute monograph, December 2005, p. 26-27, http://www.strategicstudiesinstitute.army.mil/pdffiles/pub639.pdf.

Table 16.4. Currently Designated Foreign Terrorist Organizations and BW				
Islamic State of Iraq and the Levant (formerly al-Qa'ida in Iraq)	NO	**Unknown**		Emerging group with enormous resources. Reports of chemical munitions use (chlorine, phosphine, and mustard).[2552,2553,2554,2555,2556] One individual member had a laptop with over 35,000 files dedicated to Jihad, a few of which discussed BW.[2557] Concern over alleged looting of biological laboratories in Syria.[2558] In 2014, DHS secretary Jeh Johnson stated that his service had "seen no specific credible intelligence that ISIS is attempting to use any sort of disease or virus to attack our homeland."[2559]
Islamic Jihad Union (IJU)	NO	NO		
Harakat ul-Jihad-i-Islami/Bangladesh (HUJI-B)	NO	NO		
al-Shabaab	NO	NO		
Revolutionary Struggle (RS)	NO	NO		
Kata'ib Hizballah (KH)	NO	NO		
Harakat ul-Jihad-i-Islami (HUJI)	NO	NO		
Tehrik-e Taliban Pakistan (TTP)	NO	NO		
Jundallah	NO	NO		

[2552] Tom Coghlan, Catherine Philp, Ammar Shamary, "Jihadists unleash chemical weapons in battle for Tikrit," *The Times*, March 14, 2015, <http://www.thetimes.co.uk/tto/news/world/middleeast/article4381521.ece>.

[2553] "Chlorine bomb attacks by jihadists are growing threat to the UK, warns chemical warfare expert," *The Independent*, May 25, 2015, http://www.independent.co.uk/news/uk/home-news/chlorine-bomb-attacks-by-jihadists-are-growing-threat-to-the-uk-warns-chemical-warfare-expert-10274947.html.

[2554] Phosphine, chemical formula PH_3, is used as a fumigant, but is toxic if inhaled. Ibid; also see: Sajila Saseendran, "Ministry mulls banning 'killer' pesticide," *Khaleej Times*, September 2, 2014, http://www.khaleejtimes.com/article/20140901/ARTICLE/309019899/1002.

[2555] Nabih Bulos, "Islamic State confirmed to have used mustard gas against Kurds in Syria," *The Telegraph*, August 15, 2015, http://www.telegraph.co.uk/news/worldnews/middleeast/syria/11805235/Islamic-State-confirmed-to-have-used-mustard-gas-against-Kurds-in-Syria.html.

[2556] Paul Blake, "US official: 'IS making and using chemical weapons in Iraq and Syria'," *BBC News*, September 11, 2015, http://www.bbc.com/news/world-us-canada-34211838.

[2557] Harald Doornbos, Jenan Moussa, "Found: The Islamic State's Terror Laptop of Doom," *Foreign Policy*, August 28, 2014, http://foreignpolicy.com/2014/08/28/found-the-islamic-states-terror-laptop-of-doom/.

[2558] Ari Soffer, "Experts Warn of Al Qaeda Biological Weapons Threat," *Israel National News*, October 16, 2013, http://www.israelnationalnews.com/News/News.aspx/172897#.VXddiUbrJ-A.

[2559] "Use of Ebola virus as bioterror weapon highly unlikely: Experts," *Homeland Security News Wire*, November 11, 2014, http://www.homelandsecuritynewswire.com/dr20141111-use-of-ebola-virus-as-bioterror-weapon-highly-unlikely-experts.

Table 16.4. Currently Designated Foreign Terrorist Organizations and BW				
Army of Islam (AOI)	NO	NO		
Indian Mujahedeen (IM)	**YES**	NO		A 2010 email claiming to be from "Indian Mujahedeen (Assam)" threatened biological warfare unless its demands were met.[2560]
Jemaah Anshorut Tauhid (JAT)	NO	NO		Splinter group from Jemaah Islamiyah.
Abdallah Azzam Brigades (AAB)	NO	NO		
Haqqani Network (HQN)				
Ansar al-Dine (AAD)	NO	NO		
Boko Haram	NO	NO		
Ansaru	NO	NO		
al-Mulathamun Battalion	NO	NO		
Ansar al-Shari'a in Benghazi	NO	NO		
Ansar al-Shari'a in Darnah	NO	NO		
Ansar al-Shari'a in Tunisia	NO	NO		
Ansar Bayt al-Maqdis	NO	NO		
al-Nusrah Front	**Unknown**	**Unknown**		Emerging group. Concern over alleged looting of biological laboratories in Syria.[2561]
Mujahidin Shura Council in the Environs of Jerusalem (MSC)	NO	NO		

[2560] "Extremists Warn of Biological Strike in India," *Nuclear Threat Initiative Global Security Newswire*, October 4, 2010, http://www.nti.org/gsn/article/extremists-warn-of-biological-strike-in-india/.

[2561] Ari Soffer, "Experts Warn of Al Qaeda Biological Weapons Threat."

16.10 Detailed History of Known Terrorist Biological Weapons Programs

Based on the research presented below, three international terrorist groups (Al Qaeda, Jemaah Islamiyah, and Aum Shinrikyo) and two domestic terrorist groups (Rajneesh Cult, R.I.S.E.), have sought a biological weapons capability intended for mass casualty attacks. Only the Rajneesh Cult launched a successful, albeit rudimentary, biological weapons attack. The Rashneeshees hoped to sicken, but did not seek to kill, many individuals. Aum Shinrikyo wished to cause deaths, but failed in all of its biological weapons attack attempts. Of the five groups, only Al Qaeda is likely to be pursuing a biological weapons capability at the present time. Jemaah Islamiyah's membership, including its core leadership and all known BW-program members, has been decimated in recent years due to a string of arrests. Aum Shinrikyo's WMD program has been dismantled, and key members are in jail. As for R.I.S.E., it is a long-defunct group that only had two core members.

Finally, one apparent "false positive" case exists in older reports in which a group that initially was flagged as had interest in BW appear to have been incorrect, or at least remain unsubstantiated by publicly available information. However, open source literature references that the Weather Underground group (disbanded in 1976) had attempted to blackmail a homosexual officer to obtain incapacitating agents from the US Army's Fort Detrick BW research center, with the ultimate goal being to incapacitate (but not kill) individuals by poisoning a US city water supply.[2562] A thorough review of this case by John V. Parachini in a volume edited by Jonathan B. Tucker concludes: "contrary to the conventional wisdom, the Weather Underground probably did not seek to acquire or employ biological or chemical weapons."[2563]

Summarizing these case studies, only a select few terrorist groups have demonstrated their intent to use biological weapons to cause mass casualties in the past. These programs involved few core members (from two to fourteen), and engaged even fewer individuals with advanced training in the life sciences. All four groups attempted to obtain or obtained virulent pathogenic strains by acquiring seed cultures from laboratories by leveraging some form of insider access. Of these groups, only Al Qaeda's efforts in acquiring BW are thought to be ongoing.

The following vignettes review the aforementioned five confirmed bioterrorist programs:

16.10.1 Al Qaeda

Al Qaeda has worked on biological weapons with the intent to cause mass casualties since before the 9/11 attacks. Significant uncertainties remain as to the group's achievements.

16.10.1.1 Motivation and Intent to Use

Official Al Qaeda statements underline the group's intent to use biological weapons against US citizens. An internal account of discussions occurring within the group's ruling body, the Majlis al-Shura, makes clear that in 1998, the leadership debated the utility of pursuing WMD.[2564] The supporters of WMDs in that debate won out, apparently through rhetoric that a WMD capability would be needed to prevent America and Israel from employing WMDs; in essence, they argued that WMD was necessary for deterrence.[2565] Bin Laden announced his strong support for WMD in a December 22, 1998 interview, which specifically mentioned biological weapons:

2562 John V. Parachini, "The Weather Underground (1970)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan B. Tucker (Cambridge: Belfer Center for Science and International Affairs, 2000), p. 43, 47.

2563 Ibid.

2564 Sammy Salama, Lydia Hansell, "Does Intent Equal Capability? Al-Qaeda and Weapons of Mass Destruction," *Nonproliferation Review* 12, no. 3 (November 2005): p. 625-626, p. 650fn.84.

2565 Ibid.

"Muslim scholars have issued a fatwa against any American who pays taxes to his government. He is our target, because he is helping the American war machine against the Muslim nation."[2566]

Bin Laden then stated, specifically mentioning biological weapons:

"It is very strange: if America has all the mass-destruction weapons, that is nothing. If the Jewish state has the same weapons, that is OK. But if a Muslim state like Pakistan tries to defend itself against the Hindu hegemony in South Asia, everything should be done to prevent it from doing so. We don't consider it a crime if we tried to have nuclear, chemical, biological weapons. Our holy land is occupied by Israeli and American forces. We have the right to defend ourselves and to liberate our holy land."[2567]

After the 9/11 attacks and the expulsion of Al Qaeda from Afghanistan, the group's rhetoric embraced the notion of first strike. On June 12, 2002, Al Qaeda spokesman Abu Ghaith produced a three-part article titled "In the Shadow of the Lances," that included the following passage:

"We have the right to kill four million Americans – two million of them children – and to exile twice as many and wound and cripple hundreds of thousands. Furthermore, it is our right to fight them with chemical and biological weapons, so as to afflict them with the fatal maladies that have afflicted the Muslims because of the [Americans'] chemical and biological weapons."[2568]

On May 21, 2003, radical cleric Nasir al Fahd issued a fatwa permitting the use of WMDs just before his arrest.[2569] In "A Treatise on the Legal Status of Using Weapons of Mass Destruction against Infidels," Nair al Fahd attempted to justify: 1) the use of techniques that would not kill individuals "in a good manner," such as acts to "bomb, destroy, burn or flood;" 2) the killing of children and women; and 3) the killing of Muslims.[2570,2571] Nasir al Fahd's statement was harnessed by Al Qaeda's ideologues. For instance, he is cited by Ayman Zawahiri, the current leader of Al Qaeda, in the latter's 2008 book *Exoneration*.[2572]

Al Qaeda's *Encyclopedia of Jihad*, an eleven volume training manual, reportedly includes instructions for the manufacture and use of chemical and biological weapons; if true, the topic's inclusion in the manual reinforces the evidence that the group sees the use of these weapons as justified and desirable.[2573] A 2012 article published by the Yemen branch of Al-Qaeda (AQAP) in the group's jihadist magazine *Inspire* confirms that this interpretation remains current, since it emphasized that "the use of poisons of chemical

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2566 Jamal Ismail, "I Am Not Afraid of Death," *Newsweek*, January 10, 1999, http://www.newsweek.com/i-am-not-afraid-death-165374.

2567 Ibid.

2568 The Middle East Media Research Institute (MEMRI), "Contemporary Islamist Ideology Authorizing Genocidal Murder," Special Report No.25, January 27, 2004, http://www.memri.org/report/en/0/0/0/0/0/0/1049.htm#_ednref21.

2569 Rolf Mowatt-Larssen, "Islam and the Bomb: Religious Justification For and Against Nuclear Weapons," Working paper, Harvard Kennedy School Belfer Center for Science and International Affairs, January 2011, p. 38, http://belfercenter.hks.harvard.edu/files/uploads/Islam_and_the_Bomb-Final.pdf.

2570 Ibid.

2571 Sammy Salama, Edith Bursac, "Jihadist Capabilities and the Diffusion of Knowledge," *Jihadists and Weapons of Mass Destruction*, eds. Gary Ackerman, Jeremy Tamsett (Boca Raton: CRC Press, 2009).

2572 International Research Center (IRC), "Zawahiri Tries to Clear Name, Explain Strategy," Transnational Security Issues Report, April 21, 2008, p.4, retrieved at: https://fas.org/irp/eprint/zawahiri.pdf.

2573 Sammy Salama, Lydia Hansell, "Does Intent Equal Capability? Al-Qaeda and Weapons of Mass Destruction," p. 618; Nick Fielding, "Encyclopedia of Terror: Revealed: The bloody pages of Al-Qaeda's killing manual," *The Sunday Times of London*, April 11, 2001.

and biological weapons against population centers is allowed and strongly recommended due to the effect on the enemy."[2574]

16.10.1.2 Program History

A former senior CIA official, Rolf Mowatt-Larssen, who is publicly described as having "led the US government's efforts to determine whether al Qaeda had WMD capabilities," provides several key details in the following account of Al Qaeda's BW efforts.[2575] Wherever possible, open source reporting has been used to corroborate, expand upon, or challenge his account.

A systematic pre-9/11 Al Qaeda biological weapons program was launched and headed by Ayman Zawahiri, following the merger of the latter's Egyptian Islamic Jihad group with Al Qaeda Central in early 1998.[2576] Ayman Zawahiri was a long-time proponent of biological weapons and is today the leader of Al Qaeda Central.

More specifically, Zawahiri communicated electronically with Mohammed Atif, the head of Al Qaeda's Military Committee (killed in 2001), on the setup of a chemical and biological weapons project named "al-Zabadi."[2577] A message by Zawahiri to Atif dated April 15, 1999 pushed for the development of biological weapons, arguing that "the destructive power of these weapons is no less than that of nuclear weapons" in terms of causing mass casualties.[2578] The message made clear that Zawahiri was looking to hire an expert: "I would like to emphasize what we previously discussed—that looking for a specialist is the fastest, safest, and cheapest way. Simultaneously, we should conduct a search on our own."[2579] The proposed start-up budget was outlined as $2000-4000 USD.[2580]

Zawahiri proceeded with this plan and recruited Rauf Ahmed, a mid-level Pakistani government biologist, into Al Qaeda's biological weapons efforts that same year.[2581] Ahmed's tasking included the setup of a laboratory in Kandahar, Afghanistan.[2582] Rauf Ahmed corresponded with Zawahiri, initially noting trouble with finding a *B. anthracis* strain. One letter read, verbatim: "Unfortunately, I did not find the required culture of B. anthrax – i.e., pathogenic."[2583] Whether Rauf Ahmed ever managed to obtain a pathogenic strain is not known based only on open source information.[2584]

The following passage from historian Christopher Andrew, based on internal MI5 documents made available to the researcher, fill in some gaps:

2574 Gary A. Ackerman, Lauren E. Pinson, "An Army of One: Assessing CBRN Pursuit and Use by Lone Wolves and Autonomous Cells," *Terrorism and Political Violence* 26, no. 1 (2014): p. 229-230;
"Al-Qaeda Magazine Urges Chemical, Biological Strikes Against Foes," *NTI Global Security Newswire*, May 3, 2012, http://www.nti.org/gsn/article/al-qaeda-magazine-urges-chemical-biological-strikes-us/.

2575 Rolf Mowatt-Larssen, "Al Qaeda's Pursuit of Weapons of Mass Destruction," *Foreign Policy*, January 25, 2010, http://foreignpolicy.com/2010/01/25/al-qaedas-pursuit-of-weapons-of-mass-destruction/.

2576 Ibid.

2577 Alan Cullison, Andrew Higgings, "Computer in Kabul holds chilling memos," *The Wall Street Journal*, December 31, 2001.

2578 Alan Cullison, "Inside Al-Qaeda's Hard Drive," *The Atlantic*, September 2004, http://www.theatlantic.com/magazine/archive/2004/09/inside-al-qaeda-s-hard-drive/303428/.

2579 Ibid.

2580 Ibid.

2581 Rolf Mowatt-Larssen, "Al Qaeda Weapons of Mass Destruction Threat: Hype or Reality?," p. 14.

2582 Ibid.

2583 Joby Warrick, "Suspect and A Setback In Al-Qaeda Anthrax Case," *The Washington Post*, October 31, 2006, p.3, http://www.washingtonpost.com/wp-dyn/content/article/2006/10/30/AR2006103001250_3.html.

2584 Whether Al Qaeda in general ever obtained a pathogenic strain of *B. anthracis* has not been disclosed in open sources, as discussed below in the current document.
Judith Miller, "U.S. Has New Concerns About Anthrax Readiness," *The New York Times*, December 28, 2003, http://www.nytimes.com/2003/12/28/national/28ANTH.html?pagewanted=1.

> *"In September 2000 the Pakistani microbiologist Rauf Ahmad attended a conference in Britain on dangerous pathogens, where he sought samples from other delegates as well as help in obtaining a bioreactor and cell counter.*[2585] *The Service* [MI5] *was alerted to his activities and a search of his luggage on departure from the UK revealed £13,000, which he claimed was 'to buy equipment', documents detailing his contacts (including UK companies) and a copy of his CV. The CV revealed that Ahmad had a PhD from a university in Pakistan, had attended earlier conferences in Britain in 1997 and 1999 and had published scientific papers on anthrax. Security Service officers visited the UK companies with which Ahmad had made contact and they broke off their dealings with him. Ahmad's visits to Britain had much greater significance than was apparent at the time. Their purpose only became clear after 9/11, from documents recovered by US forces in Afghanistan in 2001. Among the documents was correspondence between 'Abu Mohamed' and 'Abu Ibrahim' about procurement of equipment, cultures and training for BW production. 'Abu Mohamed' was quickly identified as UBL's* [Osama bin Laden's] *deputy, Ayman al Zawahiri. 'Abu Ibrahim' took longer to track down. References in the correspondence to his foreign travels, attendance at conferences in the UK and attempts to procure dangerous pathogens, however, were discovered to match exactly the information on Ahmad in Security Service files."*[2586]

Yazid Sufaat was recruited to work on the Al Qaeda program after Rauf Ahmed was hired. Accounts vary as to exactly when and why this occurred, but Sufaat was brought in no later than early 2001.[2587,2588,2589] The leader of Jemaah Islamiyah, an Al Qaeda-allied group based in Indonesia, presented Yazid Sufaat to Zawahiri.[2590] Yazid Sufaat was an ex-Malaysian Army captain with a biochemistry degree from a US university (California State University-Sacramento).[2591] Following this introduction, Zawahiri entrusted Sufaat with acquiring and preparing a sample of *B. anthracis* for production.[2592] Sufaat embarked on this work at a hospital laboratory in Kandahar.[2593] Zawahiri kept Ahmed's and Sufaat's endeavors compartmentalized and neither knew of the other's existence.[2594] This was either good tradecraft, as Rolf Mowatt-Larssen alleges, or simply the result of having fired Rauf over the latter's constant requests for money and dubious loyalty to the group before hiring Sufaat, as most other sources hold.[2595] One month before the 9/11 attacks in 2001, Ayman Zawahiri inspected Ahmed's laboratory.[2596] Zawahiri was also

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2585 Rauf Ahmad is sometimes used instead of Rauf Ahmed in other accounts. See for example: George Tenet, *At the Center of the Storm: My Years at the CIA* (New York: HarperTorch, 2007), p. 278.

2586 Christopher Andrew, *Defend the Realm: The Authorized History of MI5* (New York: Vintage Books, 2009), p. 807-808.

2587 Rolf Mowatt-Larssen's account says this occurred in early 1999, as part of the setup of a "second, parallel network." René Pita and Rohan Gunaratna, who interviewed intelligence service personnel who arrested and interrogated Ahmed, state this occurred in 2000, when Zawahiri grew dissatisfied with Ahmed's work. Finally, the 9/11 Report states that "Sufaat did not start on the al Qaeda biological weapons program until after JI's December 2000 church bombings in Indonesia, in which he was involved." Rolf Mowatt-Larssen, "Al Qaeda Weapons of Mass Destruction Threat: Hype or Reality?,"

2588 René Pita, Rohan Gunaratna, "Revisiting Al-Qa'ida's Anthrax Program," *CTC Sentinel* Vol. 2 Issue 5, May 2009, p. 2, p.2fn.21, <https://www.ctc.usma.edu/posts/revisiting-al-qaida%E2%80%99s-anthrax-program>

2589 The National Commission on Terrorist Attacks Upon the United States, *The 9/11 Commission Report*, p.490fn.23, http://www.9-11commission.gov/report/.

2590 Rolf Mowatt-Larssen, "Al Qaeda's Pursuit of Weapons of Mass Destruction."

2591 Maria Ressa, "Reports: Al Qaeda operative sought anthrax," *CNN*, October 10, 2003, http://edition.cnn.com/2003/WORLD/asiapcf/southeast/10/10/alqaeda.anthrax/.

2592 Rolf Mowatt-Larssen, "Al Qaeda's Pursuit of Weapons of Mass Destruction."

2593 Rolf Mowatt-Larssen, "How to Get Terrorists to Talk," *The National Interest*, February 18, 2015, p.2, http://nationalinterest.org/feature/how-get-terrorists-talk-12270?page=2.

2594 Rolf Mowatt-Larssen, "Al Qaeda's Pursuit of Weapons of Mass Destruction."

2595 See footnote 21. Rolf Mowatt-Larssen, "Al Qaeda Weapons of Mass Destruction Threat: Hype or Reality?," p. 14-15; René Pita, Rohan Gunaratna, "Revisiting Al-Qa'ida's Anthrax Program," *CTC Sentinel* Vol. 2 Issue 5, May 2009, p. 2, p.2fn.21, https://www.ctc.usma.edu/posts/revisiting-al-qaida%E2%80%99s-anthrax-program.

2596 Rolf Mowatt-Larssen, "Al Qaeda's Pursuit of Weapons of Mass Destruction."

briefed by Sufaat on the latter's efforts on cultivating a pathogenic *B. anthracis* strain.[2597]

The subsequent US outing of the Taliban in Afghanistan disrupted the efforts described above. Ahmed was detained in Pakistan and Sufaat was arrested in Malaysia in December 2001.[2598] Pakistan cut off FBI's access to Rauf Ahmed in 2003, and he is now free.[2599]

Sensitive site exploitation of Al Qaeda camps in Afghanistan following the ousting of the Taliban unearthed evidence of the group's biological weapons program. Ahmed's laboratory was uncovered; Rolf Mowatt-Larssen's 2015 account describes it as having been "crude" and used to store purchased equipment.[2600] A US defense department spokesperson briefed the press on September 14, 2002 with photographs showing a centrifuge for liquid separation and a dryer said to have been discovered at a BW laboratory in Kandahar; although publicly unconfirmed, based on the description given this was probably Ahmed's.[2601] Moreover, one Al Qaeda site in Afghanistan whose name and location has not been disclosed held over 20 old research articles from UK journals that together "provided a method for isolating, culturing, identifying, and producing bacteria, including *Bacillus anthracis* and *Clostridium botulinum*."[2602]

Exploitation of Al Qaeda camps in Afghanistan also revealed some degree of operative training in biological warfare. Two training camps, the Durante and Tarnak Farms, reportedly provided basic training to operatives on biological weapons matters; however, since details have not been made public, these allegations may be restricted to toxins.[2603] These camps were run by chemist Abu Khabab al-Masri and by Abu Musab al-Suri.[2604] Both were proponents of the use of weapons of mass destruction (WMD) against the United States.[2605] Training manuals written by Abu Khabab al-Masri "that contain instructions for making chemical and biological weapons [...] were recovered by US forces in Afghanistan."[2606] Abu Musab al-Suri was captured in 2005 and Abu Khabab al-Masri was killed in 2008.[2607]

16.10.1.3 Capability Assessment

Whether any *B. anthracis* was produced by Al Qaeda, and whether Al Qaeda obtained the necessary *B. anthracis* seed cultures to do so, remains unclear. One individual that CIA believed was involved in Al Qaeda's BW program was captured and subjected to what the Senate Select Committee on Intelligence

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2597 Ibid.

2598 Joby Warrick, "Suspect and A Setback in Al-Qaeda Anthrax Case," *The Washington Post*, October 31, 2006, http://www.washingtonpost.com/wp-dyn/content/article/2006/10/30/AR2006103001250.html.
Maria Ressa, "Reports: Al Qaeda operative sought anthrax."

2599 Joby Warrick, "Suspect and a Setback in Al-Qaeda Anthrax Case."

2600 Rolf Mowatt-Larssen, "How to Get Terrorists to Talk," p.2.

2601 The equipment pictured could not be independently assessed in the present report, because as the media article cited below notes: "The Department of Defense refused to make available the photos of the dryer and the centrifuge it said came from the lab, or any of the other photos and slides discussed at today's briefing. In response to a reporter's question, the senior official said the department had arranged the briefing in response to reporters' requests for an unclassified version of the secret briefing on these subjects that Mr. Rumsfeld had been giving." In: Judith Miller, "Lab Suggests Qaeda Planned to Build Arms, Officials Say," *The New York Times*, September 14, 2002, http://www.nytimes.com/2002/09/14/international/asia/14LAB.html.

2602 James B. Petro, David A. Relman, "Understanding Threats to Scientific Openness," *Science* 302, no. 5652 (December 12 2003): p. 1898, http://www.sciencemag.org/content/302/5652/1898/suppl/DC1.

2603 Rolf Mowatt-Larssen, "Al Qaeda Weapons of Mass Destruction Threat: Hype or Reality?" p. 13-14.

2604 Ibid.

2605 Ibid.

2606 Ibid.

2607 "Al Qaeda: Weapons expert among dead 'heroes'," *CNN*, August 3, 2008, http://www.cnn.com/2008/WORLD/asiapcf/08/03/terrorist.killed/.

has described as "harsh treatment" during the interrogation that followed.[2608] He initially said, "we never made anthrax."[2609] Once he was told that the interrogation would not stop until "he told the truth," he then stated, crying, "I made the anthrax."[2610] Prompted, he then said he was lying.[2611] Interrogators "demonstrated the penalty for lying."[2612] The individual then repeated the "I made the anthrax" claim, promptly recanted the statement, and finally re-stated the production claim.[2613] In questioning two days later, the individual stated that he had lied "only because he thought that that was what interrogators wanted."[2614] News reports on the seizure of the laboratory facility failed to clarify whether the laboratory had been used to produce biological agents.[2615] Finally, the National Research Council (NRC) of the US National Academies, in their "Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 *Bacillus anthracis* mailings," remarked:

> *"At the end of this study, the committee was provided limited information for the first time about the analysis of environmental samples for B. anthracis Ames from an undisclosed overseas site at which a terrorist group's anthrax program was allegedly located. This site was investigated by the FBI and other federal partners as part of the anthrax letters investigation. The information indicates that there was inconsistent evidence of Ames strain DNA in some of these samples, but no culturable B. anthracis."*[2616]

According to Rolf Mowatt-Larssen, the FBI took samples from Sufaat's hospital laboratory.[2617] However, the link between this sampling activity and the operation mentioned in the NRC report has not been confirmed in open sources. Since the analysis of any gathered samples has not been made public, no open source evidence is available to assess whether Al Qaeda had successfully isolated a pathogenic strain of *B. anthracis*.

Little is known regarding any Al Qaeda plans for the *B. anthracis* once produced. However, indicators of Al Qaeda interest in crop duster airplanes of uncertain reliability existed.[2618,2619,2620] Large-scale aerosol

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2608 Senate Select Committee on Intelligence, Committee Study of the Central Intelligence Agency's Detention and Interrogation Program, Foreword by Senate Select Committee on Intelligence Chairman Dianne Feinstein, Findings and Conclusions, Executive Summary, unclassified, approved December 13, 2012, updated for release April 3, 2014, declassification revisions December 3, 2014, p. 82fn.442, http://www.intelligence.senate.gov/sites/default/files/publications/CRPT-113srpt288.pdf.

2609 Ibid.

2610 Ibid.

2611 Ibid.

2612 Ibid.

2613 Ibid.

2614 Ibid.

2615 Compare: "The lab had been abandoned by Al Qaeda before production began, officials said." In: Judith Miller, "Lab Suggests Qaeda Planned to Build Arms, Officials Say."
With: "U.S. officials said the evidence neither establishes nor rules out that al Qaeda completed manufacture." In: Barton Gellman, "Al Qaeda Near Biological, Chemical Arms Production," *Washington Post*, March 23, 2003, http://www.washingtonpost.com/wp-dyn/content/article/2006/06/09/AR2006060900918.html.

2616 National Research Council of the National Academies, *Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Bacillus Anthracis Mailings* (Washington: The National Academies Press, 2011), p. 8, 72.

2617 Rolf Mowatt-Larssen, "How to Get Terrorists to Talk," p.2.

2618 Sammy Salama, Lydia Hansell, "Does Intent Equal Capability? Al-Qaeda and Weapons of Mass Destruction."

2619 Julian Borger, "Cropdusters grounded in poison alert," *The Guardian*, September 23, 2001, http://www.theguardian.com/world/2001/sep/24/afghanistan.terrorism9

2620 "The FBI reviewed a list of some 11,000 agricultural aircraft provided by the Federal Aviation Administration, according to documents provided to the Sept. 11 commission. Working from that list, agents interviewed and did background checks on 3,028 operators and owners of the planes." In:
"FBI Checking Crop-Dusting Planes and Pilots, Still Worried About Possible Terror Use," *Police One*, April 22, 2004, http://www.policeone.com/terrorism/articles/85144-FBI-Checking-Crop-Dusting-Planes-and-Pilots-Still-Worried-About-Possible-Terror-Use/.

dissemination may therefore have been a possibility, a threat which FBI took extremely seriously.[2621]

Few details have surfaced regarding Al Qaeda's post-2001 BW efforts. One exception was a document titled "Terror Franchise: The Unstoppable Assassin, TECHS Vital role for its success," found during the 2011 raid that killed Bin Laden. It was prepared by a senior Al Qaeda member, Abu-Salih al Somali, and must have been written sometime after 2009 given the events referenced in the text.[2622]

This document places particular emphasis on the use of poisons and biological weapons, which it classes as "toxicants." The document, written in broken English, is addressed to "Engineers, Doctors, Biologists, Pharmacists, researchers, hobbyists, Handymen and women, experimenters, discoverers, The courageous, Experts in all fields, Amateurs, and all of you who care and realize that you are part of an Ummah."[2623] It calls for assistance from these "techs" in producing and disseminating knowledge and know-how on, *inter-alia*, "how to be able to make **death**, in its **explosions** form- especially (the Oxidizer part of it) and **toxicants** in an easy, practical and improvised way anywhere on earth..." [Emphasis and punctuation in original][2624]

The document reminds the reader that "Americans and their NATO allies' citizens" are to be targeted in a campaign to cause "nonstop, unpredicted, invisible sudden death," and gives as an example the use of cyanide or ricin on products sold by supermarkets and restaurants.[2625] The document ends with a detailed list of military topics the author is requesting the "techs" to research and subsequently share their findings through instruction manuals and videos. The following list of requests, emphasized as "immediately needed," is found under the "toxicants" request section:

1. *Actual improvised production and testing of Cyanides, Ricin.(immediately needed),*
2. *Preparation and testing (rabbits is ok) of any lethal (delayed and immediate) ingested toxicants.*
3. *On Camera production of any of war gases (Phosgene, VX, etc.). Look at Nbk file and scientific principles of improvised home warfare. (onsite production apparatus also).*
4. *Actual production and testing of Biological toxicants (Anthrax, Botulinum, clostridium, endotoxins, Exotoxins, etc.),*
5. *Production of (HCl) or whatever is needed in the production of toxicants,*
6. *Any other options that can be used as toxicant... plants, etc.... detailed, local names pictures, incidents, cultivation... Insects... etc.... read scientific principles of improvised home warfare volume 2,5,6.*
7. *Bacteria based weapons.. how? detail. Any other practical options.*

2621 Rolf Mowatt-Larssen, "Al Qaeda's Pursuit of Weapons of Mass Destruction."
2622 David Francis, "Al Qaeda's Blueprint For How To Start a Homegrown Terror Franchise," *Foreign Policy*, May 20, 2015, http://foreignpolicy.com/2015/05/20/al-qaedas-blueprint-for-how-to-start-a-homegrown-terror-franchise/.
2623 Office of the Director of National Intelligence, Bin Laden's Bookshelf," http://www.dni.gov/index.php/resources/bin-laden-bookshelf?start=1.
Retrieved under the "Now Declassified Material" folder. Abu-Salih Al Somali, "Terror Franchise: The Unstoppable Assassin, TECHS Vital role for its success," http://www.dni.gov/files/documents/ubl/english/Terror%20Franchise.pdf.
2624 Ibid.
2625 Ibid.

8. *Airborne substance that when sprayed in small quantity or mixed (tablet form) with water, tranquilizes the entire inhabitants of a hall or plane. And its antidote.*[2626]

This document confirms the group's continued interest in obtaining and using biological weapons to cause mass civilian deaths. At the same time, it highlights the group's paucity of expertise in the matter, at least as of 2009.

16.10.2 Jemaah Islamiyah

Jemaah Islamiyah is a Southeast Asian terrorist group that has been allied with Al Qaeda since 1998.[2627] The group has been in sharp decline, although it remains on the US list of Foreign Terrorist Organizations.[2628,2629,2630] Jemaah Islamiyah had a joint biological warfare program with Al Qaeda organized by Riduan Isamuddin and run by Sufaat. Riduan Isamuddin was Jemaah Islamiyah's director of operations.[2631] He oversaw the group's financing, led Jemaah Islamiyah's regional policy-making organ, and organized Al Qaeda's regional operations.[2632] Riduan Isamuddin suggested and organized the transfer of Sufaat to Al Qaeda's BW program. When US operations in Afghanistan began in October 2001, Sufaat fled Afghanistan for Bogor, Indonesia.[2633] He then sought to set up a new BW program in-country, but failed to recruit a microbiologist at an Indonesian institute.[2634,2635,2636] He was captured in December 2001; Isamuddin was apprehended in 2003.[2637] A Jemaah Islamiyah manual indicating interest in chemical and biological weapons was reportedly discovered in the Philippines in 2003.[2638]

16.10.3 Aum Shinrikyo

The Japan-based millenarian cult and terrorist group, Aum Shinrikyo, embarked on a WMD program in 1990, intending to cause mass casualties and precipitate the apocalypse.[2639] The group's WMD network was dismantled following their March 1995 sarin nerve agent attack in the Tokyo Subway and the subsequent arrest of top Aum leaders. However, Aum Shinrikyo remains on the US list of Foreign

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2626 Ibid.
2627 The National Commission on Terrorist Attacks Upon the United States, *The 9/11 Commission Report*, p. 151.
2628 Fouad Pervez, "Jemaah Islamiyah," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 370.
2629 National Counterterrorism Center, "Jemaah Islamiyah (JI)," September 2013, http://www.nctc.gov/site/groups/ji.html.
2630 U.S. Department of State, "Foreign Terrorist Organizations," <http://www.state.gov/j/ct/rls/other/des/123085.htm
2631 United Nations Security Council Committee pursuant to resolutions 1267 (1999) and 1989 (2011) concerning Al-Qaida and associated individuals and entities, "QDi.087 Nurjaman Riduan Isamuddin," March 28, 2011, http://www.un.org/sc/committees/1267/NSQDi087E.shtml.
2632 Ibid.
2633 Judith Miller, "U.S. Has New Concerns About Anthrax Readiness," *The New York Times*, December 28, 2003, http://www.nytimes.com/2003/12/28/us/us-has-new-concerns-about-anthrax-readiness.html
2634 Ibid.
2635 Maria Ressa, "Reports: Al Qaeda operative sought anthrax," *CNN*, October 10, 2003, http://edition.cnn.com/2003/WORLD/asiapcf/southeast/10/10/alqaeda.anthrax/.
2636 René Pita, Rohan Gunaratna, "Revisiting Al-Qa'ida's Anthrax Program,"
2637 Rolf Mowatt-Larssen, "Al Qaeda Weapons of Mass Destruction Threat: Hype or Reality?" p. 28.
2638 Christopher Torchia, "Experts: Bioterrorism Should Worry Asia," *Associated Press*, March 25, 2006.
2639 Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," *Center for a New American Security*, December 2012, p. 18-20, http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf.

Terrorist Organizations, perhaps in part because of concerning reports in 2000 that the cult was regrouping.[2640,2641,2642]

16.10.3.1 Program History

The group's biological weapons program was based on *B. anthracis*, alongside a toxin weapons program focused on botulinum toxin and a chemical weapons program focused primarily but not exclusively on the nerve agent sarin.[2643] Certain Aum Shinrikyo members voiced a passing interest in Ebola virus as a weapon, although no evidence that the group attempted to acquire a pathogen sample exists.[2644,2645]

The group's biological weapons team was composed of about ten individuals, led by a graduate-trained molecular biologist named Seiichi Endo.[2646] Endo had taken courses in molecular biology and genetic engineering at the PhD level at the Viral Research Center at Kyoto University, but did not complete enough coursework to obtain a doctorate degree.[2647] This team drew upon cult rank-and-file members to carry out equipment purchases. The team's BW endeavor was sustained by the group's significant infrastructure and finances, which allowed for the liberal purchase of laboratory equipment and the acquisition of reference texts.[2648] The cult specifically targeted scientists, engineers, and technicians that could be of use for the group's weapons programs in their recruitment campaigns.[2649]

After an abortive phone call to the US Centers for Disease Control and Prevention, the group decided against attempting to purchase a strain from a US culture collection for fear of being discovered.[2650] Endo reportedly attempted and failed to steal a *B. anthracis* strain from a laboratory, after which an insider with

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2640 U.S. Department of State, "Foreign Terrorist Organizations," http://www.state.gov/j/ct/rls/other/des/123085.htm.

2641 David E. Kaplan, "Aum Shinrikyo," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan B. Tucker (Cambridge:Belfer Center for Science and International Affairs.

2642 Sara Daly, John Parachini, William Rosenau, *Aum Shinrikyo, Al Qaeda, and the Kinshasa Reactor: Implications of Three Case Studies for Combating Nuclear Terrorism* (Santa Monica: RAND, 2005), p. 12, http://www.rand.org/content/dam/rand/pubs/documented_briefings/2005/RAND_DB458.pdf.

2643 Raymond A. Zilinskas, *Biological Warfare: Modern Offense and Defense* (Boulder: Lynne Rienner Publishers, Inc., 2000), p. 79-81.

2644 The reports that the group attempted to obtain an Ebola strain in Zaire in October 1992 were not born out by the current authoritative study on Aum Shinrikyo by Danzig et al. that enjoyed unprecedented access to imprisoned top members of the group.
Richard Danzig, Marc Sageman, Terrance Leighton, Lloyd Hough, Hidemi Yuki, Rui Kotani, Zachary M. Hosford, "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," Center for a New American Security, December 2012.
http://www.cnas.org/files/documents/publications/CNAS_AumShinrikyo_SecondEdition_English.pdf.

2645 For leader statements supporting the passive interest in Ebola, see:
D. W. Brackett, *Holy Terror: Armageddon in Tokyo* (New York: Weatherhill, 1996), p. 102;
Amy E. Smithson, "Rethinking the Lessons of Tokyo," in *Ataxia: The Chemical and Biological Terrorism Threat And The US Response*, ed. Amy E. Smithson, Stimson Report 35, October 9, 2000, p. 74, 74fn.12. <http://www.stimson.org/books-reports/ataxia-the-chemical-and-biological-terrorism-threat-and-the-us-response/>

2646 Endo completed graduate courses, but dropped out before finishing his graduate degree, hence the use of the term "graduate-trained."
Amy E. Smithson, "Rethinking the Lessons of Tokyo," p. 75;
Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," p. 13, 23.

2647 Amy E. Smithson, "Rethinking the Lessons of Tokyo," p. 75fn.15.

2648 Ibid.

2649 For instance, Funihiro Joyu tried to recruit Russian chemical engineers with sarin nerve agent production experience.
Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," p. 52.

2650 Ibid.

sympathies for the cult provided him access to a non-pathogenic vaccine strain.[2651] The individual who allegedly did so was never found.[2652] Independent interviews with two core Aum Shinrikyo BW program members confirmed that they knew the strain obtained was non-pathogenic.[2653] Endo had promised them that he could use "genetic engineering" to modify the strain to be pathogenic.[2654] Richard Danzig et al., in their seminal study of the group's WMD efforts, noted that Endo could have attempted to exploit a known viable method to make a non-pathogenic strain pathogenic.[2655] However, the evidence that this method was attempted remains speculative. In any case, Endo proclaimed success, and the group attempted several ineffectual attacks with *B. anthracis*.[2656] Danzig et al. concluded by stating, "to this day we nor the leaders of Aum Shinrikyo know whether Endo possessed a fully virulent strain of *B. anthracis* and was unable to conserve it, or whether he conserved it but could not amplify it, or whether he never achieved it at all."[2657]

16.10.3.2 Capability Assessment

The *B. anthracis* production lines were crude. The first production method tried was a liquid line, set up in 1992 at a cult property in Kameido, Tokyo.[2658] The cult apparently relied on 200-liter drums to act as fermenters, with ten drums used for a production run.[2659] No attempt was made at separate the pathogenic culture from the growth media through liquid purification; the resultant slurry was used directly.[2660] In 1993, following failed attacks with the liquid mixture, the group attempted to dry the product and disseminate the resultant powder.[2661] As before, no attempt was made to separate the growth media from the pathogens.[2662]

Regarding delivery system capabilities, Aum Shinrikyo maintained a vehicle with a mounted spray-dryer system for the biological and toxin programs, but the spray system was highly defective.[2663] It was employed once unsuccessfully with the group's non-pathogenic *B. anthracis*.[2664] The spray system was manufactured by the cult themselves, because the group's leader did not want to wait the two months needed to order and receive a sprayer from a European firm.[2665]

16.10.4 Rajneesh Cult

The Rajneesh Cult was started by an individual calling himself Bhagwan Shree Rajneesh in India in the 1960s.[2666] By 1981, the cult had moved to Wasco County, Oregon (US).[2667] By spring 1984, the cult faced

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2651 No further details are known.
Richard Danzig et al., "Aum Shinrikyo: Insights into how terrorists develop biological and chemical weapons, second edition," p. 23.
2652 Ibid.
2653 Ibid.
2654 Ibid.
2655 Ibid.
2656 Ibid.
2657 Ibid.
2658 Ibid.
2659 Ibid.
2660 Ibid.
2661 Ibid.
2662 Ibid.
2663 Ibid.
2664 Two failed attempts with botulinum toxin had previously been launched using the same platform. Ibid.
2665 Ibid.
2666 W. Seth Carus, "Rajneeshees," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons, 2011), p. 383-384.
2667 Ibid.

significant legal troubles, including land conflicts and immigration law violation investigations.[2668] A highly influential member, Ma Anand Sheela, decided to devise a plan whose goal was the replacement of two commissioners with cult members in the November 1984 election.[2669] Ma Anand Puja, a senior cult member close to Sheela and a registered nurse, was to organize a biological weapons attack to sicken locals and thereby prevent them from voting.[2670] They hoped that doing so would enable the cult's candidates to win the election despite their unpopularity in the local community.

16.10.4.1 Program History

A major "trial run" was carried out in September 1984, when *S. typhimurium* was used to contaminate at least ten restaurant salad bars in The Dalles, Oregon.[2671] No individuals died, but at least 751 people fell ill as a result of the attack.[2672,2673] When the cult realized that they had no chance of winning the local elections, they abandoned the planned November attack.[2674] The September attack was misattributed by health agencies as caused by unsanitary practices by restaurant workers for over a year.[2675] The incident only came to light because of a major rift between Bahgwan Rajneesh and Seela and Puja; the two women fled the US camp, and Bahgwan Rajneesh retaliated by publicizing their actions.[2676]

At least one biological attack was carried out before the major September attack.[2677] On August 29, 1984, two Wasco County commissioners were given water deliberately tainted with *S. typhimurium*, and both fell ill.[2678] Reports, based on admissions made by Rajneesh members, allege that other cult attacks took place prior to August 1984, namely: one attack against the Wasco County Courthouse, attacks against schools, nursing homes, and political gatherings, one attack against The Dalles' water supply, and one attack against a supermarket.[2679,2680] These incidents are unconfirmed, since these alleged attacks apparently did not cause illnesses or were not carried out by disobeying members, and since members may have had a desire to exaggerate Puja's wrongdoings given the aforementioned internal conflict and a general dislike for Puja.[2681,2682]

16.10.4.2 Capability Assessment

Roughly fourteen individuals were associated with the cult's biological weapons program: three or four individuals were directly involved in culturing the Salmonella-causing pathogen for use in the September attack, while seven or eight appear to have spread the biological agent (both teams had some overlap).[2683]

2668 Ibid.
2669 Ibid.
2670 Ibid.
2671 Ibid.
2672 Thomas J, et al. (1997) "A Large Community Outbreak of Salmonellosis Caused by Intentional Contamination of Restaurant Salad Bars." *Journal of the American Medical Association* 278, no. 5: 389-395, http://www.cdc.gov/phlp/docs/forensic_epidemiology/Additional%20Materials/Articles/Torok%20et%20al.pdf.
2673 W. Seth Carus, "The Rajneeshees (1984)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan Tucker Cambridge: The MIT Press.
2674 W. Seth Carus, "Rajneeshees," p. 534-535.
2675 Ibid.
2676 Ibid.
2677 Ibid.
2678 Ibid.
2679 Ibid.
2680 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, Working Paper (Washington: Center for Counterproliferation Research, National Defense University, February.
2681 Ibid.
2682 W. Seth Carus, "The Rajneeshees (1984)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan Tucker Cambridge: The MIT Press.
2683 Ibid.

The cult used common microbiological techniques to produce the desired quantities of agent: the pathogen was grown on agar in Petri dishes and incubated.[2684] Puja ordered the *S. typhimurium* sometime between October 1, 1983 and February 29, 1984 from a medical supply company.[2685] Puja had reportedly also ordered cultures of *S. typhi*, *Salmonella paratyphi*, *Francisella tularensis*, and other undisclosed pathogens from the American Type Culture Collection, using her status as a nurse at the Rashneeshee's state-licensed medical laboratory.[2686] The decision to use *S. typhimurium* rather than these or other agents was presumably made based on the desire to keep the attack covert, the desire to make people sick but not kill them and the relative ease of culturing the organism, although no accounts of Puja's final decision exists.[2687]

16.10.5 RISE

R.I.S.E. was a small domestic eco-radical group.[2688] The group's founders, the college students Stephen J. Pera and Allen C. Schwandner, were arrested on January 18, 1972.[2689] They had formed a group they called R.I.S.E., and reportedly planned to contaminate Chicago's municipal water system with *Salmonella typhi* (causative agent of typhoid fever).[2690] Precisely what the acronym R.I.S.E. stood for is not known.[2691] Two new recruits turned on the two founders and reported the plot to the police.[2692] The following account of the group's activities come from publications by W. Seth Carus, who remains the only researcher to have extensively studied this case to date.

16.10.5.1 Motivation and Intent to Use

Pera was an adopted child with a troubled childhood who repeatedly did not get along with others.[2693] For instance, he was asked to leave a microbiology program sponsored by the International Foundation for Microbiology due to conflicts with other students.[2694] Pera and Schwandner believed that mankind was destroying the planet, and that the only way to prevent this was to wipe out the human race, except for a chosen small group of like-minded individuals.[2695] Despite reports to the contrary, the group had no neo-Nazi or racist tendencies.[2696] Although Pera and Schwandner eventually fled to Cuba, they did not appear to have a particular affinity for communist countries, part of their planning involved striking the Soviet Union and China, because they feared that these countries would capitalize on the group's planned destruction of the Western powers.[2697] The group's knowledge of eco-radical theory was rather primitive, suggesting that R.I.S.E. was mostly a byproduct of their inability to adapt to society.[2698]

[2684] W. Seth Carus, "The Rajneeshees (1984)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan Tucker Cambridge: The MIT Press.
[2685] Ibid.
[2686] Ibid.
[2687] Ibid.
[2688] W. Seth Carus, "RISE: A Case Study," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas Hoboken: John Wiley & Sons.
[2689] Ibid.
[2690] Ibid.
[2691] Ibid.
[2692] Ibid.
[2693] Ibid.
[2694] Ibid.
[2695] Ibid.
[2696] Ibid.
The R.I.S.E. case appears to be the exact same incident as one reported in passing in some secondary sources involving a supposed far-right group called the "Order of the Rising Sun." The plot details and timeline are identical. R.I.S.E. was not a far-right group, but as Seth Carus has noted is sometimes flagged as such; there must have been some initial media confusion, and this is perhaps what led to the "Order of the Rising Sun" story.
[2697] Ibid.
[2698] Ibid.

16.10.5.2 Program History

The group had obtained a range of pathogens and considered aerosol and food contamination as alternative dissemination pathways to the planned water supply attack.[2699] Pera obtained *S. typhi* and *N. meningitides* from a hospital microbiology laboratory where he volunteered.[2700] *C. diphtheria* and *S. sonnei* were also obtained by the group through some unknown means.[2701] The group used this laboratory to culture pathogens. In December 1971, Pera was kicked out of the lab for having tried to acquire controlled substances illegally, and the hospital authorities destroyed his samples.[2702] The group relocated its activities to Mayfair College laboratories until the police arrested the two leaders.[2703]

16.10.5.3 Capability Assessment

In W. Seth Carus' judgement, "although R.I.S.E. appears to have been motivated to conduct a mass-casualty attack with biological weapons, it lacked the scientific and technical expertise to carry it out."[2704] The water supply contamination scheme would have failed had it been carried out, and although the group talked about aerosol dissemination, the members had no relevant knowledge or experience.[2705] Schwandner had no technical expertise. He enrolled at Mayfair College to study the humanities, but rapidly stopped attending any of his classes.[2706] Pera was largely self-taught in microbiology, with only some low-level work experience to complement limited and incomplete coursework from Mayfair College.[2707] His cultures were found to have contained several organisms, demonstrating that he lacked the skill necessary to prevent culture contamination.[2708] Pera was the only member with any scientific experience.[2709]

16.11 Other Terrorist/Extremist Groups Linked in Some Fashion to Biological Weapons

As discussed in Section 16.10, only five terrorist groups have sought a biological weapons capability intended for mass casualty attacks. Another 14 groups have been linked in some fashion with biological terrorism.[2710] Four of these groups made apparently-empty threats. The count includes animal rights extremist groups (two such groups are included). The count however excludes any groups involved with toxin-only acts or threats. Very few information is available in open sources on several of the cases below. The cases (numbered) are as follows:

2699 Ibid.
2700 Ibid.
2701 The group was originally suspected of having Botulinum toxin, but "subsequent tests [...] indicated that the two did not have any botulinum toxin." W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 102.
2702 Ibid.
2703 Ibid.
2704 Ibid.
2705 Ibid.
2706 Ibid.
2707 The number of courses Pera signed up for and actually completed is unclear. Pera did not finish at least one course, and appears to have signed on for at most two other courses.
2708 W. Seth Carus, "RISE: A Case Study," *Encyclopedia of Bioterrorism Defense, 2nd Edition*, eds. Rebecca Katz, Raymond A. Zilinskas (Hoboken: John Wiley & Sons.
2709 Ibid.
2710 In part based on W. Seth Carus' "Appendix A: List of Cases:"
W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, Working Paper (Washington: Center for Counterproliferation Research, National Defense University, February 2001 Revision), p. 179-198.
Note that the "World Islamic Front for Fighting Jews and Christians," noted in one case, is an Al Qaeda alias.
U.S. Department of State, Bureau of Counterterrorism, "Country Reports on Terrorism 2013 - Chapter 6. Foreign Terrorist Organizations," April 2014, <http://www.state.gov/j/ct/rls/crt/2013/224829.htm>.

1- The eco-radical group "Dark Harvest" took *B. anthracis*-contaminated soil from Gruinard Island (a then-contaminated British military World War II site used to test *B. anthracis* bombs) and spread it on the grounds of Porton Down in 1981.[2711] The soil did contain *B. anthracis*, although no harm resulted from the act.[2712] No further acts were attributed to this group, and it is presumed defunct.

2, 3, 4- Three ethno-nationalist groups have reportedly used biological agents to enhance the effectiveness of conventional explosive attacks. Government forces have claimed that the FARC (Colombia), ELN (Colombia), and NPA (Philippines) groups spiked explosive devices with feces to cause sepsis, in what appears to be a modern take on the Viet Cong punji stick technique.[2713,2714,2715] NPA has denied these claims.[2716] All three groups remain active and are designated as Foreign Terrorist Organizations by the US Department of State.

5- A Palestinian group (unknown) was reportedly caught in a counterfeiting scheme whereby expired eggs contaminated with salmonella were stamped with counterfeit stamps indicating their acceptability to be eaten, and sold.[2717] Israeli news reporting on the group's capture in May 2000 implied that this was deliberately done to sicken Israelis.

6- The German-based, now-defunct, Red Army Faction (RAF) reportedly maintained a botulinum toxin laboratory in Paris, France until it was uncovered in October 1980.[2718] However, a recent review of this case has cast doubt on parts of the underlying story, and German authorities apparently remain convinced that "no evidence whatsoever [exists] that members of the 'RAF' had planned or prepared an attack using biological agents."[2719,2720]

7,8- Two animal rights radical groups have used the threat of HIV contamination to heighten fear. A spokesman for the Animal Liberation Front (ALF) claimed in 1993 that bombs planted in the UK by members of the collective had been purposefully tainted with HIV, but authorities dismissed this account.[2721] Similarly, the "Justice Department" mailed razors to fur retailers in Canada in 1996 which they claimed were covered with HIV-infected blood, although whether they had really done so is not known.[2722]

9- The "Counter Holocaust Lobbyists of Hillel" sent agar and *B. cereus* in a petri dish apparently labelled

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2711 Porton Down was Britain's main biodefense and chemical warfare defense establishment, and previously the center running Britain's biological weapons program. W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 58.

2712 Ibid.

2713 Pablo Esteban Parra Gallego, "IEDs: A Major Threat for a Struggling Society," *The Journal of ERW and Mine Action* 13, no. 3 (Winter 2009), <http://www.jmu.edu/cisr/journal/13.3/specialreport/gallego/gallego.shtml>;

2714 Mariano C. Bartolome, Maria Jose Espona, "Chemical and Biological Terrorism in Latin America: The Revolutionary Armed Forces of Colombia," *The ASA Newsletter* 03-5, no. 98 (October 31, 2003), http://www.asanltr.com/newsletter/03-5/articles/035e.htm

2715 "Philippine Army finds human feces, snake venom in wounded soldiers' wounds," *Mindanao Examiner*, September 4, 2013, http://mindanaoexaminer.com/philippine-army-finds-human-feces-snake-venom-in-wounded-soldiers-wounds/.

2716 Ibid.

2717 Jason Pate, Gavin Cameron, "Covert Biological Weapons Attacks against Agricultural Targets: Assessing the Impact against U.S. Agriculture," BCSIA Discussion Paper 2001-9, ESDP Discussion Paper ESDP-2001-05, John F. Kennedy School of Government, Harvard University, August 2001, p.8, http://belfercenter.ksg.harvard.edu/files/covert_biological_weapons_attacks_against_agricultural_targets.pdf.

2718 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 156-157.

2719 Ibid.

2720 The review in question is:
Terence Taylor, Tim Trevan, "The Red Army Faction (1980)," *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, ed. Jonathan B. Tucker (Cambridge: MIT Press, 2000), p. 107-113.

2721 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 76.

2722 Ibid.

"*anthracs"* [SIC] and *"Yersinia*" to a Jewish organization in Washington in 1997.[2723,2724] The package contained a hate latter that further misrepresented the petri dish as containing a "chemical warfare" agent.[2725] Whether this was an anthrax hoax, or whether the group thought the package contained *B. anthracis*, is unknown. No further acts were attributed to this group, and it is presumed defunct.

10- The Chinese government alleges that Emeti Yakuf, an alleged terrorist connected to the East Turkistan Islamic Movement, threatened to use biological and chemical weapons to disrupt the 2008 Olympics held in China, and that he trained group members on making poisons.[2726] This individual was reportedly killed in a 2012 US drone strike in Pakistan.[2727]

11 to 14- Another four groups have reportedly threatened to use a biological agent, but did not specify what type of agent, and are not known to have possessed biological agents. These were: Chechen separatists (in general), the "Republic of Texas" group, the Al-Aqsa Martyrs Brigade, and the "Indian Mujahedeen (Assam)."[2728,2729,2730]

16.12 The Islamic State of Iraq and the Levant (ISIL)

ISIL (also called ISIS or Daesh) is a Sunni violent Islamist group currently in control of territory in Syria, Iraq, and Libya. It is fighting against the Iraqi government's mostly-Shia forces, and is a major player in the Syrian civil war.[2731] It seeks to establish and expand its own state: a caliphate with its leader, Abu Bakr al-Baghdadi, as caliph.[2732,2733] Public estimates of the group's fighting strength vary tremendously, from a low of 20,000 to a high of 200,000 fighters.[2734,2735,2736] As explained in a report generated by the United Nations' Analytical Support and Sanctions Monitoring Team for the United Nations Security

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2723 Ibid.

2724 The B'nai B'rith International Jewish Monthly, Volume 111, (1996), p. 67, https://books.google.com/books?id=V--3AAAAIAAJ&q=anthracis+Yersinia+Counter+Holocaust+Lobbyists+of+Hillel&dq=anthracis+Yersinia+Counter+Holocaust+Lobbyists+of+Hillel&hl=en&sa=X&ved=0CC8Q6AEwA2oVChMI98TMwLKIxgIVOEaMCh0gNAC0.

2725 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 111; Matthew Dorf, "Questions lingering after bizarre mailing to B'nai B'rith," *J Weekly*, May 2, 1997, http://www.jweekly.com/article/full/5673/questions-lingering-after-bizarre-mailing-to-b-nai-b-rith/.

2726 "'Eastern Turkistan' terrorists identified," *China Daily*, October 21, 2008, http://www.chinadaily.com.cn/china/2008-10/21/content_7126503.htm.

2727 Declan Walsh, Eric Schmitt, "Militant Leader Believed Dead in Pakistan Drone Strike," *The New York Times*, August 24, 2012, http://www.nytimes.com/2012/08/25/world/asia/us-drone-strikes-kill-18-in-pakistan.html?_r=1.

2728 W. Seth Carus, *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900*, p. 107-109, 186.

2729 Michael Moodie, Markus Binder, "Jihadists and Chemical Weapons," *Jihadists and Weapons of Mass Destruction*, eds. Gary Ackerman, Jeremy Tamsett (Boca Raton: CRC Press, 2009), p. 143

2730 "Extremists Warn of Biological Strike in India," *Nuclear Threat Initiative Global Security Newswire*, October 4, 2010, http://www.nti.org/gsn/article/extremists-warn-of-biological-strike-in-india/.

2731 "Islamic State- The Pushback," *The Economist*, March 21, 2015, <http://www.economist.com/news/briefing/21646752-sustaining-caliphate-turns-out-be-much-harder-declaring-one-islamic-state-not#correction>.

2732 United Nations Security Council, "Letter dated 13 November 2014 from the Chair of the Security Council Committee pursuant to resolutions 1267 (1999) and 1989 (2011) concerning Al-Qaida and associated individuals and entities addressed to the President of the Security Council," S/2014/815, November 14, 2014, p.6-7, para. 7, 12, http://www.securitycouncilreport.org/atf/cf/%7B65BFCF9B-6D27-4E9C-8CD3-CF6E4FF96FF9%7D/s_2014_815.pdf.

2733 بيروت - أ ف ب [Beirut – AFP], "داعش" يعلن "الخلافة الإسلامية" ويبايع البغدادي ["ISIS" declares the "Islamic caliphate," pledges allegiance to al-Baghdadi], *Alhayat*, June 29, 2014, http://www.alhayat.com/Articles/3292478

2734 United Nations Security Council, "Letter dated 13 November 2014 from the Chair of the Security Council Committee pursuant to resolutions 1267 (1999) and 1989 (2011) concerning Al-Qaida and associated individuals and entities addressed to the President of the Security Council," p. 8, para. 14.

2735 "Islamic State formations comprise up to 70,000 gunmen- Chief of Russia's General Staff," *TASS*, December 10, 2014, http://tass.ru/en/world/766237.

2736 Patrick Cockburn, "War with Isis: Islamic militants have army of 200,000, claims senior Kurdish leader," *The Independent*, November 16, 2014, http://www.independent.co.uk/news/world/middle-east/war-with-isis-islamic-militants-have-army-of-200000-claims-kurdish-leader-9863418.html.

Council, part of the uncertainty stems from the lack of clarity as to "whether all those fighting with ISIL […] have actually pledged loyalty to the group, or are in allied militia groups, or are opportunistically aligning with ISIL, or have been forced to fight."[2737] Compounding these issues, all available estimates are now dated, having been issued at the end of 2014. Estimates of the number of individuals living in areas under ISIL control are also uncertain: cited figures include "about 8 million" and "10 million people."[2738,2739]

The group's leadership is dominantly Iraqi, given that ISIL evolved from Abu Musab al-Zarqawi's Al Qaeda in Iraq. Zarqawi had serious strategic disagreements with Osama bin Laden's Al Qaeda central, starting in February 2004, over the former's desire to heavily target Iraq's Shia population and thereby incite sectarian violence.[2740] Al-Zarqawi was subsequently killed in a US airstrike in June 2006.[2741] Abu Bakr al-Baghdadi took control of the group in 2010, and the group changed names in 2013 and 2014.[2742,2743] Reconciliation efforts between ISIL and Al Qaeda central failed, and the latter formally disassociated itself from ISIL in February 2014.[2744]

The aforementioned U.N. report noted that ISIL is "particularly well-armed given its access to extensive supplies of heavy weapons seized from the Government of Iraq," and "has fighters with experience in conventional warfare who are well-versed on a range of weapons systems, including the use of tanks and artillery."[2745] Group propaganda has displayed numerous heavy weapons in use, including anti-tank missile systems.[2746,2747,2748,2749]

[2737] United Nations Security Council, "Letter dated 13 November 2014 from the Chair of the Security Council Committee pursuant to resolutions 1267 (1999) and 1989 (2011) concerning Al-Qaida and associated individuals and entities addressed to the President of the Security Council," p. 8, para. 14.

[2738] The 10 million estimate was given by Peter Maurer, president of the International Committee of the Red Cross, in March 2015. Stephanie Nebehay, "Islamic State-controlled parts of Syria, Iraq largely out of reach: Red Cross," *Reuters*, March 13, 2015, <http://www.reuters.com/article/2015/03/13/us-mideast-crisis-syria-icrc-idUSKBN0M921N20150313>.

[2739] The "about 8 million" estimate is given in: "Islamic State- The Pushback," *The Economist*.
For an analysis of issues with generating these figures, see: Frank R. Gunter, "The ISIL Invasion of Iraq: Economic Winners and Losers," *Foreign Policy Research Institute*, July 2014, <http://www.fpri.org/articles/2014/07/isil-invasion-iraq-economic-winners-and-losers>.

[2740] Emily Hunt, "Zarqawi's 'Total War' on Iraqi Shiites Exposes a Divided among Sunni Jihadists," The Washington Institute PolicyWatch #1049, November 15, 2005, <http://www.washingtoninstitute.org/policy-analysis/view/zarqawis-total-war-on-iraqi-shiites-exposes-a-divide-among-sunni-jihadists>.

[2741] Ellen Knickmeyer, Jonathan Finer, "Insurgent Leader Al-Zarqawi Killed in Iraq," *The Washington Post*, June 8, 2006, p.1-2, <http://www.washingtonpost.com/wp-dyn/content/article/2006/06/08/AR2006060800114.html>.

[2742] Initially to the "Islamic State in Iraq and al-Sham," in April 2013; subsequently to the "Islamic State," in June 2014. Jessica D. Lewis, "Al-Qaeda in Iraq Resurgent: The Breaking The Walls Campaign, Part I," *Institute for the Study of War*, September 2013, p. 9, <http://www.understandingwar.org/sites/default/files/AQI-Resurgent-10Sept_0.pdf>.

[2743] United Nations Security Council, "Letter dated 13 November 2014 from the Chair of the Security Council Committee pursuant to resolutions 1267 (1999) and 1989 (2011) concerning Al-Qaida and associated individuals and entities addressed to the President of the Security Council," p. 7 para. 11, 12.

[2744] Liz Sly, "Al-Qaeda disavows any ties with radical Islamist ISIS group in Syria, Iraq," *The Washington Post*, February 3, 2014, https://www.washingtonpost.com/world/middle_east/al-qaeda-disavows-any-ties-with-radical-islamist-isis-group-in-syria-iraq/2014/02/03/2c9afc3a-8cef-11e3-98ab-fe5228217bd1_story.html.

[2745] United Nations Security Council, "Letter dated 13 November 2014 from the Chair of the Security Council Committee pursuant to resolutions 1267 (1999) and 1989 (2011) concerning Al-Qaida and associated individuals and entities addressed to the President of the Security Council," p. 14 para. 37.

[2746] "Open Syria @OpenSyria" [Twitter handle / Pseudonym], "IS TOW use geo-located ~2.3km NE of Palmyra, documented in "The Raid of Abu Malik A-Tamimi […]" *Twitter*, June 8, 2015, https://twitter.com/OpenSyria/status/607885537115987968

[2747] "Open Syria @OpenSyria" [Twitter handle / Pseudonym], "#IS Kornet ATGM deployed at the #Hasakah prison, reported tank kill […]," *Twitter*, June 2, 2015, https://twitter.com/OpenSyria/status/605641058446278656.

[2748] "Open Syria @OpenSyria" [Twitter handle / Pseudonym], "9M14M Malyutka (perhaps Iranian I-Raad) ATGM among #IS spoils in new Wilayat #Hama release […]," *Twitter*, June 1, 2015, <https://twitter.com/OpenSyria/status/605408366068760576>.

[2749] "The Islamic State's spring offensive: al-Suklua," *Oryx Blog*, May 23, 2015, http://spioenkop.blogspot.com/2015/05/the-islamic-states-spring-offensive-al.html.

The group has co-opted, recruited, or coerced numerous specialists. In one notable case of co-optation, Bashar al-Assad regime's technical experts maintaining the Euphrates dam near Raqqa have remained on site on the government's payroll, allegedly in exchange for the continued operation of the ISIL-controlled dam.[2750,2751,2752] ISIL used engineers to operate the oil refineries it initially controlled, although these operations have been heavily disrupted by coalition airstrikes against ISIL-controlled refineries and related transport convoys.[2753,2754] A February 2015 report by the US Financial Action Task Force noted that ISIL controlled "numerous oil fields from which it continue[d] to extract oil for its own use [and] its own refining," even though the group lacked the "resources and technical capacities" to fully exploit these resources.[2755] The group has also demonstrated its chemical engineering capabilities through the smuggling of chemicals such as phosphate. The possibility of such an event was raised by the FATF report authors, who remarked that the Akashat Phosphate Mine and the Al-Qaim (sulfuric acid and phosphoric acid) Manufacturing Plant had reportedly fallen under ISIL control.[2756] By June 2015, an anonymous analyst had released to the public satellite imagery showing the Al-Qaim facility pre- and post-ISIL control, demonstrating that hundreds of tons of phosphate had been drained.[2757] ISIL members have generated propaganda specifically calling for specialists; one such appeal read, "we need engineers, we need doctors, we need professionals. Every person can contribute something."[2758] ISIL members recruited Western medical professionals, in part through propaganda portraying "really good medical service" in occupied areas and calling upon medical students to join in building a new society.[2759]

The possibility that the group could harness its resources and human technical base to develop and employ unconventional weapons has been raised in public reports numerous times.[2760] Several claims that ISIL is employing readily available dangerous chemicals as chemical weapons in Syria have been published in the media.[2761] Strong open source information supporting these claims emerged from a series of mortar shell attacks that occurred in June and July 2015. A 120-millimeter mortar shell modified to disseminate a chemical agent, "most probably chlorine," was found in Kurdish positions.[2762] It was

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2750 Yezid Sayigh, "The War Over Syria's Gas Fields," *Carnegie Endowment for International Peace*, June 8, 2015, http://carnegieendowment.org/syriaincrisis/?fa=60316.

2751 Danya Chudacoff, "'Water war' threatens Syria lifeline," *Al Jazeera*, July 7, 2014, <http://www.aljazeera.com/news/middleeast/2014/07/water-war-syria-euphrates-201475764032066 3.html>.

2752 Jan Ali, "Euphrates Dam… another victim of Syrian war," *ARA News*, December 6, 2014, <http://aranews.net/2014/12/euphrates-dam-another-victim-syrian-war/>.

2753 Fazel Hawramy, Shalaw Mohammed, Luke Harding, "Inside Islamic State's oil empire: how captured oilfields fuel Isis insurgency," *The Guardian*, November 19, 2014, <http://www.theguardian.com/world/2014/nov/19/-sp-islamic-state-oil-empire-iraq-isis>.

2754 Financial Action Task Force (FATF), "Financing of the Terrorist Organisation Islamic State in Iraq and the Levant (ISIL)," February 2015, p. 13, <http://www.fatf-gafi.org/media/fatf/documents/reports/Financing-of-the-terrorist-organisation-ISIL.pdf>.

2755 Financial Action Task Force (FATF), "Financing of the Terrorist Organisation Islamic State in Iraq and the Levant (ISIL)," p. 13.

2756 Ibid, p. 16.

2757 "Not a spy @fintriswolf" [Twitter handle / Pseudonym], "#Iraq :Unpublished Imagery. #ISIS has removed hundreds of tons of phosphate from this facility […]," *Twitter*, June 7, 2015, <ttps://twitter.com/fintriswolf/status/607466224927186944>.

2758 Liezel Hill, Scott Deveau, Gerrit De Vynck, "Canadians from Calgary to Timmins heed ISIL's tweets," *Bloomberg*, October 23, 2014, <http://www.bloomberg.com/news/articles/2014-10-23/canadians-from-calgary-to-timmins-heed-islamic-state>.

2759 Katrin Bennhold, "Young Medics Were Lured by Briton to Join ISIS," *The New York Times*, July 17, 2015, <http://www.nytimes.com/2015/07/18/world/europe/young-medics-were-lured-by-briton-to-join-isis.html?rref=world/middleeast>.

2760 See for example: David Albright, Serena Kelleher-Vergantini, Sarah Burkhard, "Syria's Unresolved Nuclear Issues Reemerge in Wake of ISIL Advance and Ongoing Civil War," Institute for Science and International Security – Imagery Brief, June 30, 2015, p. 1-7, <http://isis-online.org/uploads/isis-reports/documents/Syria_June_30_2015_Final.pdf>.

2761 C. J. Chivers, "ISIS Has Fired Chemical Mortar Shells, Evidence Indicates," *New York Times*, July 17, 2015, <http://www.nytimes.com/2015/07/18/world/middleeast/islamic-state-isis-chemical-weapons-iraq-syria.html?smid=tw-share>.

2762 Ibid.

analyzed by a military ordnance expert working for Sahan Research in partnership with Conflict Armament Research.[2763] Another similar mortar shell, analyzed by an expert from Conflict Armament Research, apparently contained phosphine and had also been fired against Kurdish forces.[2764] US intelligence agencies reportedly have concluded that ISIL used mustard agent in subsequent attacks in Syria and Iraq.[2765,2766]

Despite these alarming trends, no credible, open source evidence exists confirming whether ISIL is seeking a biological weapons capability. A journalistic piece in *Foreign Policy* described the contents of an alleged ISIL member's laptop hard drive, obtained by journalists for *Foreign Policy*, was found to contain over 35,000 files dedicated to Jihad, a few of which discussed BW.[2767] However, whether the alleged owner intended to act on the BW files is not known. Moreover, the contents as described have no grounding in technicality and read like extracts from extremist "weapons cookbook" literature available on the World Wide Web. For instance, the snippet of text from the file made public was: "Use small grenades with the virus, and throw them in closed areas like metros, soccer stadiums, or entertainment centers. Best to do it next to the air-conditioning. It also can be used during suicide operations."[2768] The text mischaracterizes *Y. pestis* as a virus and seemingly provides no instructions on how to create a "small grenade" that would disseminate the agent successfully.

16.13 Biosafety and Biosecurity at US Research Laboratories

The requirements that govern US laboratory operations primarily derive from four sources: statutes, regulations, guidance, and contracts. (Appendix V: Section 16.11 lists all governing documents that are relevant to the biosecurity risk assessment.) Many security requirements are regulatory while biosafety requirements are either contractual, associated with inspections of regulatory programs, or voluntary. Furthermore, standards in guidance documents often are considered de facto requirements, especially when needed for facility certification, regulatory compliance, liability protection, and compliance with funding contracts or terms and conditions of grant awards.

To build a realistic picture of defense measures, both requirements and practice are considered. Recognizing that institutions may be subject to state, local, and tribal requirements and institutional policies all of which vary, the evaluation of defensive measures is based solely on federal governing instruments and their implementation. Indications of practice may be gleaned from guidance documents, peer-reviewed literature, other publically available sources, and interviews of officials representing institutions conducting relevant research. However, no overarching documentation of industry best practices exists, even though the desire to create such a mechanism has been voiced.

Figure 16.3 highlights required and voluntary measures at for non-select agent high containment laboratories, select agent laboratories, and Tier 1 select agent laboratories. Some of the voluntary measures, such as institutional threat assessment teams and surveillance of animal facilities, apply to non-high containment laboratories.

[2763] Ibid.

[2764] Phosphine, chemical formula PH_3, is used as a fumigant, but is toxic if inhaled. Ibid; also see: Sajila Saseendran, "Ministry mulls banning 'killer' pesticide," *Khaleej Times*, September 2, 2014, <http://www.khaleejtimes.com/article/20140901/ARTICLE/309019899/1002>.

[2765] Nabih Bulos, "Islamic State confirmed to have used mustard gas against Kurds in Syria," *The Telegraph*, August 15, 2015, <http://www.telegraph.co.uk/news/worldnews/middleeast/syria/11805235/Islamic-State-confirmed-to-have-used-mustard-gas-against-Kurds-in-Syria.html>.

[2766] Paul Blake, "US official: 'IS making and using chemical weapons in Iraq and Syria'," *BBC News*, September 11, 2015, <http://www.bbc.com/news/world-us-canada-34211838>.

[2767] Harald Doornbos, Jenan Moussa, "Found: The Islamic State's Terror Laptop of Doom," *Foreign Policy*, August 28, 2014, <http://foreignpolicy.com/2014/08/28/found-the-islamic-states-terror-laptop-of-doom/>.

[2768] Ibid.

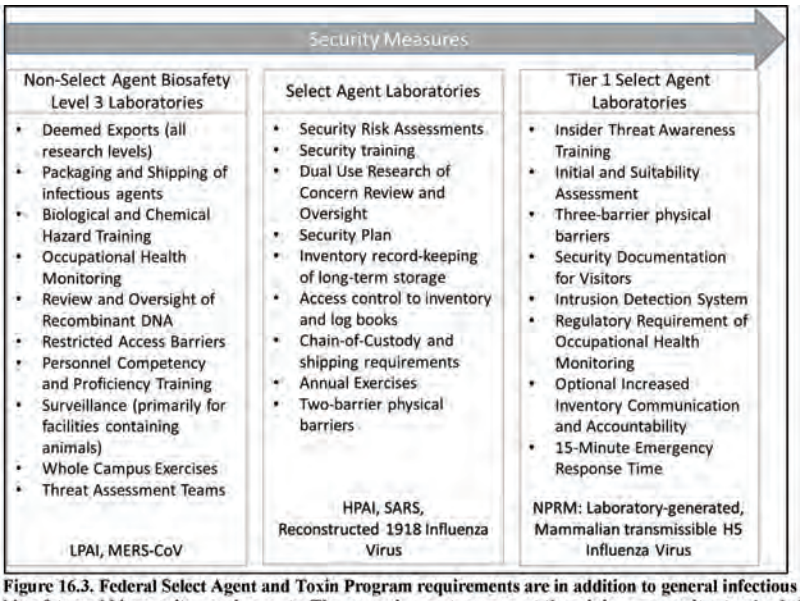

Figure 16.3. Federal Select Agent and Toxin Program requirements are in addition to general infectious agent biosafety and biosecurity requirements. These requirements represent the minimum security standards for institutions. Many institutions implement additional safety and security measures, some of which are included in the figure.

16.13.1 Biosafety Levels, Select Agents, and Risk Assessments

The laboratory operating environment can be broadly characterized using a tiered, agent-specific, and experiment-specific framework. This framework draws on two classification systems: 1) Biosafety Levels (BSLs) specifying containment, access, and security measures and 2) the Federal Select Agent Program that requires special safety and security precautions for designated agents.[2769,2770] Under this framework, an institution carries out a risk assessment before all planned experiments with pathogens to identify the safety and, if applicable, the security risk of the experiment. The Federal Select Agent Program (FSAP) requires that institutions and individuals seeking access to agents on the Biological Select Agents and Toxins (BSAT) list be approved by the FSAP and under conditions specified by the FSAP. The biosafety risk assessment helps the institution implement the appropriate measures necessary to mitigate risk and comply with statutes and regulations. The Biosafety Levels, the FSAP, and the risk assessment process are described in turn below.

[2769] Federal Select Agent and Toxin Program http://selectagents.gov.

[2770] Biosafety levels were originally established in the 1970s. Current updates can be found in the latest version of the BMBL. Nancy Connell, "Biological Agents in the Laboratory- The Regulatory Issues," *Public Interest Report* [Federation of American Scientists] 64, no. 3 (Fall 2011): p. 13, http://fas.org/pubs/pir/2011fall/2011FALL-PIR-lowres.pdf.

16.13.1.1 Biosafety Levels

Biosafety Levels described in the BMBL are a means to categorize laboratory containment capabilities based on facility specifications, safety equipment, and microbiological practices. BSLs range from lowest (BSL-1) to highest (BSL-4) levels of containment.[2771,2772] Laboratories that work with experimentally infected animals require special measures at all levels compared to laboratories that do no such work. To make the distinction clear, animal labs are categorized in a similar manner, using an Animal Biosafety Level 1-4 scale that ranges from ABSL-1 (lowest containment) to ABSL-4 (highest containment).[2773] USDA further established the BSL-3-Agriculture (-Ag), BSL-3 Enhanced, and ABSL-3 Enhanced levels to describe special measures to reduce risk of environmental contamination when working with certain livestock and plant pathogens.[2774] A full description and comparison of Biosafety Levels can be found in the BMBL.[2775]

The biosafety levels describe safety-specific measures primarily intended to prevent laboratory-acquired infections and environmental release. However, certain measures also directly reduce security risks. For instance, physical barriers and access controls serve a dual safety and security purpose. Figure 16.4 below highlights some of the similarities and differences between the various biosafety levels on topics of relevance to security. The table focuses on security-related measures and does not characterize the full set of requirements for the biosafety levels. Selected biosafety measures are incorporated into broad security-related categories: physical security, surveillance and monitoring, personnel training and reliability, and emergency response.[2776]

[2771] Ibid.
[2772] U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 24.
[2773] Ibid.
[2774] Ibid.
[2775] Ibid.
[2776] These are the subset of security categories presented in section 1.9 which appear in BSL recommendations.

Security relevance	Topic	BSL-1	BSL-2	BSL-3	BSL-3 Enhanced	BSL-4	ABSL-1	ABSL-2	ABSL-3	ABSL-3 Enhanced	BSL-3-Ag	ABSL-4
Physical Security	Access control	Lab supervisor enforces institutional access control policies										
	Entry/exit requirements	N/A	Visitors must meet entry/exit requirements		BSL-3 plus change and shower rooms controlling access	BSL-3 plus locked doors, logbook, need-to-enter basis only, showers	Only those required for program or support purposes		ABSL-2 + limit access more	ABSL-3 + showers		ABSL-3 plus locked doors, logbook, need-to-enter basis only, showers
	Doors/building	Doors required	Self-closing lockable doors	BSL-2 + separate space from unrestricted traffic flow, 2 self-closing doors, restricted access	BSL-3 + emergency exit doors locked from outside	Separate building/zone, locked, alarms entry-exit/security systems on a UPS. Special double door for passing through materials	Separated from unrestricted traffic flow, inward opening, self-closing doors		ABSL-2 + double door entry	ABSL-3 + emergency exit doors locked from outside	ABSL-3 + Sealed hinges and latch/knob, gaskets on doors	Separate building/zone, locked, alarms entry-exit/security systems on a UPS. Special double door for passing through materials
	Windows	Screens	Screens (preferred not openable)	Sealed		Sealed, break-resistant	Not recommended for security reasons. If exist, break resistant. Sealing optional	ABSL-1 + sealing required				Sealed, break-resistant
	Double-door autoclave access	N/A			Access control not specified	Access to exit side of pass-through only for those authorized to enter the lab	N/A			Exit side access control not specified		Access to exit side of pass-through only for those authorized to enter the lab
	Decontamination and disposal	May be removed from facility before disposal		Preferably decontaminate in facility		Must decontaminate in laboratory	May be removed from facility before disposal	Autoclave + incineration recommended			Double door autoclave + incinerate	Must decontaminate in laboratory
	Signage	Biohazard sign, POC, Agent info (if req'd by institution)	BSL-1 + label entry/exit procedures				Biohazard sign, POC, Agent info (if req'd by institution)					
	Operational planning	N/A		Test/verify facility design/operations annually			N/A	Test/verify facility design/operations annually				
Surveillance and Monitoring	Surveillance and reporting	N/A	Surveillance & immunizations as needed			BSL-3 + system for reporting absenteeism/accidents/exposures, quarantine facility	Surveillance, immunizations, and respiratory programs as needed. Ensure medical staff know hazards.					ABSL-3 + system for reporting absenteeism/accidents/exposures, quarantine facility
Personnel training	Training (microbial practices)	N/A	Demonstrate microbiological proficiency to lab supervisor			BSL-3 + higher microbio training, lab operations training, annual updates	Not specified					BSL-3 + higher microbio training, lab operations training, annual updates
Emergency Response	Emergency protocols	N/A				Emergency preparedness plans and training required (medical, fires, natural disasters, other)	Plan for man-made or natural disasters and recovery					
	Communications	N/A				Emergency comms, access/egress plans required	N/A					Emergency comms, access/egress plans required

Figure 16.4: Security-relevant requirements for BSL-1 through BSL-4, and ABSL-1 through ABSL-4, laboratories[2777]

[2777] U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p.30-103, 343-350.

16.13.1.2 Biological Select Agents and Toxins

The list of Biological Select Agents and Toxins designate the pathogens and toxins[2778] that pose a high threat to human, animal, or plant health and, hence, require special additional biosafety and biosecurity measures.[2779] The Antiterrorism and Effective Death Penalty Act of 1996 established the Select Agent Program required restricted transfer of certain biological agents, now known as BSAT.[2780] The current Select Agents program was created through a significant expansion of this initial system in response to the September 11 attacks and the "Amerithrax" attacks of 2001.[2781,2782,2783]

The BSAT list classifies pathogens depending on the disease host (human, animal, overlap human and animal, and plant). HHS maintains a list of HHS Select Agents causing disease in humans including those on the overlap list, the US Department of Agriculture (USDA) maintains a list of Veterinary Service Select Agents causing disease in animals including those on the overlap list, and the Animal and Plant Health Inspection Service (APHIS) maintains a list of Plant Protection and Quarantine Select Agents.[2784] Although the pathogens covered by each of these lists differ, the additional safety and security requirements mandated in the relevant Parts of the US Code of Federal Regulations for select agents are functionally equivalent.[2785,2786,2787,2788] That is, although the governing agency may change depending on the pathogen considered, the security requirements for any BSAT are identical in practice. For this reason, agents on any of these three lists are typically referred to as "Biological Select Agents and Toxins," without reference to a particular list.

The Select Agent Program further designates certain Select Agents as Tier 1 Select Agents.[2789] These agents "have the potential to pose a severe threat to public health and safety" (HHS) or "pose a severe

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[2778] Regulations involving toxins are not within the scope of this report, and references that apply solely to toxins are therefore omitted.

[2779] Nancy Connell, "Biological Agents in the Laboratory- The Regulatory Issues," p. 14.

[2780] These were initially called Listed Biological Agents, and were solely agents with "the potential to pose a severe threat to public health and safety" as determined by the HHS Secretary.
Antiterrorism and Effective Death Penalty Act of 1996, Public Law 104-132, 104th Congress, Subtitle B--Biological Weapons Restrictions, Sec. 511, http://www.gpo.gov/fdsys/pkg/PLAW-104publ132/html/PLAW-104publ132.htm.

[2781] A short history can be found at the Select Agent program webpage: Centers for Disease Control and Prevention (CDC), Animal and Plant Health Inspection Service (APHIS), "History," http://www.selectagents.gov/history.html.

[2782] USA PATRIOT Act of 2001, Public Law 107-56, 107th Congress, Title VIII—Strengthening the Criminal Laws Against Terrorism, Sec. 817, http://www.gpo.gov/fdsys/pkg/PLAW-107publ56/html/PLAW-107publ56.htm.

[2783] Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Public Law 107-188, 107th Congress, Subtitle D—Criminal Penalties Regarding Certain Biological Agents and Toxins, Sec. 231, http://www.gpo.gov/fdsys/pkg/PLAW-107publ188/html/PLAW-107publ188.htm.

[2784] Pathogens can be on both the HHS and USDA Select Agents lists, and are termed Overlap Select Agents.

[2785] The American Biological Safety Association described the USDA select agents requirements as "essentially identical to the requirements for select agents regulated by HHS." A review of the relevant regulations supports this statement.
American Biological Safety Association, "Re: Federal Register Docket CDC-2012-0010," December 14, 2012, http://www.absa.org/pdf/121214ABSACommentsCDC-2012-0010.pdf.

[2786] U.S. Government Publishing Office, "Title 42: Public Health, §73.3 HHS select agents and toxins," www.ecfr.gov/cgi-bin/text-idx?SID=27b43dad6d0e40ba856cd39358931a6f&mc=true&node=se42.1.73_13&rgn=div8.

[2787] U.S. Government Publishing Office, "Title 9: Animals and Animal Products, §121.3 VS select agents and toxins," http://www.ecfr.gov/cgi-bin/text-idx?SID=e84486ed28bdb8517340f1c8b365ba9c&mc=true&node=se9.1.121_13&rgn=div8.

[2788] U.S. Government Publishing Office, "Title 7: Agriculture, §331.3 PPQ select agents and toxins," http://www.ecfr.gov/cgi-bin/text-idx?SID=a2965b1fa4298b718b9259a19efe533f&mc=true&node=se7.5.331_13&rgn=div8.

[2789] The risk-based tiering of the Select Agents lists was mandated through Executive Order 13546, Sec. 4. Executive Order 13546, "Optimizing the Security of Biological Select Agents and Toxins in the United States" (July 2010) http://www.gpo.gov/fdsys/pkg/FR-2010-07-08/pdf/2010-16864.pdf.

threat to animal health or to animal products" (USDA).[2790,2791] Tier 1 Select Agents have additional safety and security requirements that go beyond what is required for non-Tier 1 Select Agents and Toxins.

Three pathogens have additional special safety and security requirements that go beyond the Tier 1 requirements: variola (major and minor) virus, rinderpest virus, and foot-and-mouth disease virus. These pathogens are not within the scope of the current report. Therefore, the specific measures for these pathogens are not summarized below, although their special security measures have been examined during our review of the possible security landscape and in considering potential recommendations.

BSAT requirements are in addition to the general infectious agent biosafety measures (Figure 16.3). Under the current requirements, MERS-CoV and low pathogenic avian influenza (LPAI) are not BSAT. SARS-CoV, highly pathogenic avian influenza (HPAI), and recombinant 1918 influenza are Select Agents. The CDC has recently proposed categorizing laboratory-modified H5N1 influenza virus strains as Tier 1 Select Agents.[2792]

For all infectious agent research, the operating environment is primarily defined by the selection of a biosafety level. For work with a Select Agent or Tier 1 Select Agent, additional safety and security requirements will significantly affect operations.[2793] The combination of biosafety level and Select Agent status define a framework within which all security-related requirements and practices can be analyzed.

Most GoF research is conducted at various types of Level 3 containment (BSL-3, BSL-3 Enhanced, ABSL-3, BSL-3-Ag, or ABSL-3 Enhanced). Some may occur under Level 2 containment with additional respiratory protection. GoF research currently uses non-Select and Select Agents, though some recombinant influenza strains may be reclassified as Tier 1 Select Agents in the near future.

16.13.1.3 The Risk Assessment Process

The risks of a given research plan are agent-specific and experiment-specific. Risk assessments are a key part of experiment planning. Biosafety risk assessments (also known as biological risk assessments) are required for all infectious agent research following BMBL practices, and also under OSHA and NIH guidelines.[2794,2795,2796] Currently, no federal regulations explicitly require biosecurity risk assessments for research with non-Select Agents, although the BMBL provides advisory recommendations for principles

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2790 U.S. Government Publishing Office, "Title 42: Public Health, §73.3 HHS select agents and toxins," www.ecfr.gov/cgi-bin/text-idx?SID=27b43dad6d0e40ba856cd39358931a6f&mc=true&node=se42.1.73_13&rgn=div8.

2791 U.S. Government Publishing Office, "Title 9: Animals and Animal Products, §121.3 VS select agents and toxins," http://www.ecfr.gov/cgi-bin/text-idx?SID=e84486cd28bdb8517340f1c8b365ba9e&mc=true&node=se9.1.121_13&rgn=div8.

2792 Proposed regulation covers laboratory generated, mammalian, respiratory-transmissible influenza viruses containing the hemagglutinin from the A/Goose/Guangdong/1/96 lineage. Federal Register Volume 80, Number 136, Pages 42079-42084 http://www.gpo.gov/fdsys/pkg/FR-2015-07-16/html/2015-17435.htm

2793 Work with Select Agents and Toxins must still satisfy all regular biosafety requirements for infectious agent work.

2794 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 9.

2795 U.S. Code, Title 29, Chapter 15-Occupational Safety and Health, Section 654. http://www.gpo.gov/fdsys/pkg/USCODE-2010-title29/html/USCODE-2010-title29-chap15-sec654.htm.

2796 National Institutes of Health, "NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)," November 2013, http://osp.od.nih.gov/sites/default/files/NIH_Guidelines_0.pdf.

of laboratory biosecurity.[2797,2798] Select Agent regulations require the development, implementation, and regular review of a security plan, which is designed according to a site-specific risk assessment.[2799]

16.13.1.3.1 Biosafety Risk Assessments

The biosafety risk assessment process involves laboratory directors, institutional biosafety committees, biological safety professionals, institutional review boards, animal care and use committees, and animal facility directors.[2800,2801] These risk assessors choose an appropriate biosafety level for an experiment by considering the infectivity of the pathogen, the severity of the disease it causes, its transmissibility, whether the pathogen is indigenous or exotic, and the nature of the work to be conducted.[2802] The BMBL recommends "careful judgment" during the risk assessment process; underestimating risk can be dangerous, but overprescribing measures may add expense, make research more logistically difficult, and lead to noncompliance.[2803]

Baseline biosafety level recommendations for many infectious agents are provided in the BMBL and through CDC and WHO guidance.[2804] Current BMBL and CDC guidance recommends that virus propagation in cells or animals occur in Level 3 containment (Standard, Animal, Enhanced, or Agricultural) for highly pathogenic avian influenza (HPAI), recombinant 1918 influenza, non-contemporary H2N2, SARS-CoV, and MERS-CoV; Level 2 containment (Standard or Animal) is recommended for low pathogenic avian influenza (LPAI) and currently circulating seasonal influenza.[2805,2806] The BMBL also strongly recommends a thorough risk assessment is conducted before starting any experiments where pathogenic characteristics are deliberately enhanced, as specific guidance is based on an infectious agent's "capability to infect and cause disease."[2807]

In addition to these broad risk factors, the BMBL highlights specific factors to consider during biosafety risk assessments for influenza virus research. These factors are: replication in the respiratory tract in animal models; clonal purity; phenotypic stability; gene constellations; and time since a similar strain was circulating widely in nature. Although these factors do not directly apply to research with the other agents

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2797 Additional review is required for funding of some work under Dual Use Research of Concern policy (DURC). However, all GoF-relevant pathogens on the DURC list are also Select Agents.

2798 U.S. Department of Health & Human Services, "United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern," March 2012, http://www.phe.gov/s3/dualuse/Documents/us-policy-durc-032812.pdf.

2799 Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins, Animal and Plant Health Inspection Service (APHIS) Agriculture Select Agent Program, "Security Guidance for Select Agent or Toxin Facilities: 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73," July 5, 2013, http://www.selectagents.gov/resources/Security_Guidance_v3-English.pdf.

2800 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*.

2801 This system was codified for civilian research in 1974, through CDC's *Classification of Etiologic Agents on the Basis of Hazard*. U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 3.

2802 Ibid.

2803 Ibid.

2804 Ibid.
For example, CDC provided the following guidance on MERS: Centers for Disease Control and Prevention (CDC), "Middle East Respiratory Syndrome (MERS)," June 18, 2015, http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html.

2805 Whereas guidance covers preparations such as fixed samples and untreated diagnostic specimens, we focus here on cellular and animal propagation of highest interest to the GoF research community. Certain experiments may require additional safety measures (e.g. respiratory protection at BSL-2). U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 211, 224.

2806 CDC, "Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2," June 18, 2015, http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html.

2807 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 10.

of interest– including coronaviruses– they do provide insight into some of the factors generally under consideration during biosafety risk assessments.

In addition, a laboratory manager may deem additional security measures necessary for work with non-select agents that nevertheless pose a "high public health and agriculture concern," or with commercially valuable products such as vaccine candidates.[2808]

16.13.1.3.2 Biosecurity Risk Assessments

Although biosecurity risk assessments are not required for a majority of non-Select Agent research, they may still be implemented. The BMBL recommends considering adversaries, threats, and scenarios whilst developing written security plans, standard operating procedures, incident response plans, and employee training protocols.[2809] Other sources recommend considering physical security, personnel reliability, material control and accountability, and information security.[2810]

Select Agent guidance notes that risk assessments are "the cornerstone of a good security plan."[2811] Risk assessments should be performed by a team that includes the responsible official, biological safety professionals, lead investigators, facility security and operations, federal partners, and local law enforcement. This team should assess malicious actor threats, natural hazards, consequences, and particular vulnerabilities, and develop a plan to mitigate identified risks.[2812] Tier 1 Select Agents require additional security measures, but the risk assessment process is the same.

The September 2014 institutional DURC oversight policy requires research institutions to conduct a risk assessment of proposed research to determine whether it falls within the policy's definition of "dual use research of concern."[2813] This risk assessment is a three-step process: 1) determining whether the proposed research involves one of the 15 listed agents; 2) evaluating whether the proposed research involves one of seven categories of experiments; and 3) assessing the consequences of the research to determine whether it qualifies as "dual use research of concern." If proposed research is thought to have dual use potential, the principal investigator and institution are required to identify appropriate risk mitigation plans according to the responsibilities enumerated in the institutional DURC policy.[2814]

2808 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 104-105.

2809 Ibid.

2810 LouAnn C. Burnett, "Biosafety Practices Associated with Potential Agents of Biocrime and Biowarfare," *Current Protocols in Microbiology* (2006).

2811 Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins, Animal and Plant Health Inspection Service (APHIS) Agriculture Select Agent Program, "Security Guidance for Select Agent or Toxin Facilities: 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73," July 5, 2013, http://www.selectagents.gov/resources/Security_Guidance_v3-English.pdf.

2812 Ibid.

2813 United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. (2014) Accessible at http://phe.gov/s3/dualuse/documents/durc-policy.pdf. Accessed on September 8, 2015.

2814 Ibid.

16.14 Laws, Guidance, Policies, Practices, and International Agreements on Biosafety and Biosecurity

16.14.1 Types of Governing Instruments

16.14.1.1 Statutes[2815,2816,2817]

The federal statutes relevant for this assessment generally serve to prohibit certain activities, establish criminal and civil penalties to deter such acts, and delegate regulatory authority to the executive branch Applicable statues usually do not specify functional operating requirements, which are left to the regulatory authorities.[2818]

16.14.1.2 Regulations[2819,2820,2821]

Congress may empower an executive branch agency to establish and enforce regulations published in the US Code.[2822] The regulations relevant for this assessment codify functional requirements while leaving flexibility in implementation.[2823] Federal regulations that apply to laboratory work are found throughout the Code of Federal Regulations, from broad OSHA regulations on protecting workers from hazards, to regulations on handling specific pathogens established by HHS and USDA.

Different regulations fall under different executive branch authorities. The three major federal regulatory entities for GoF laboratories are the Occupational Safety and Health Administration (OSHA), the Department of Health and Human Services (HHS), and the Department of Agriculture (USDA).[2824] Other agencies, such as the Department of Transportation, Department of Commerce, and the Environmental Protection Agency are involved in smaller roles. Appendix V provides a detail list of all relevant laws and guidance.

[2815] Any "General and permanent" law passed by Congress is compiled into the *U.S. Code*.[2815] The *U.S. Code* is the statutory law of the country. In this analysis, However, it is "a rebuttable presumption that may be corrected" if one finds unrepealed acts that are not reflected in the U.S. Code. Both the *U.S. Code* and acts of Congress published in the *Federal Register* are considered.

[2816] See: Richard J. McKinney, "Basic Overview on How Federal Laws Are Published, Organized and Cited," FLICC Program on Federal Legislative Research, January 2006, p.4 http://www.llsdc.org/assets/sourcebook/federal-laws.pdf.

[2817] 1 U.S.C. § 204 http://uscode.house.gov/view.xhtml?req=granuleid:USC-prelim-title1-section204&num=0&edition=prelimU.S. Government Publishing Office http://www.gpo.gov/fdsys/browse/collection.action?collectionCode=PLAW

[2818] For example, 18 U.S.C. § 175b leaves to regulation the designation of Federal Select Agents and Toxins. 18 U.S.C. §175b http://www.gpo.gov/fdsys/pkg/USCODE-2011-title18/pdf/USCODE-2011-title18-partI-chap10-sec175b.pdf

[2819] Federal regulations are compiled in the Code of Federal Regulations (CFR). In this report, federal regulations were retrieved through the Electronic Code of Federal Regulations.

[2820] U.S. Office of the Federal Register <http://www.ofr.gov/Catalog.aspx>.

[2821] U.S. Government Publishing Office, "Electronic Code of Federal Regulations" <www.ecfr.gov>

[2822] Richard J. McKinney, "Basic Overview on How Federal Laws Are Published, Organized and Cited," FLICC Program on Federal Legislative Research, January 2006, p.1 http://www.llsdc.org/assets/sourcebook/federal-laws.pdf.

[2823] In effect, the regulations governing biological laboratories and their activities are not very prescriptive. Jennifer Gaudioso, Susan A. Caskey, LouAnn Burnett, Erik Heegaard, Jeffery Owens, Philippe Stroot, "Strengthening Risk Governance in Bioscience Laboratories," Sandia National Laboratories, SAND2009-8070, December 2009, p.37, http://www.biosecurity.sandia.gov/BioRAM/Biorisk%20Framework%20Report.pdf.

[2824] U.S. Department of Health and Human Services (HHS), Public Health Emergency (PHE), "Biosafety and Biocontainment FAQs," http://www.phe.gov/s3/faqs/Pages/biosafety.aspx.

16.14.1.3 Guidance

Statutes and regulations are often very broad, and stress functionality rather than mandating means of implementation.[2825] Federal agencies frequently issue guidance to clarify regulations, establish best practices, and provide additional optional recommendations to improve operations. Other organizations like professional societies may also issue guidance and standards with practical recommendations.[2826] Guidance documents often describe a baseline standard that all implementations of regulations must meet. While some guidance documents provide optional recommendations, they often become viewed as *de facto* requirements for regulatory compliance, certification, compliance with contracts, and/or liability protection by the regulated community.

For instance, much of the guidance governing biosafety is enforced by OSHA through the authority derived from a general employee hazards protection law. The Occupational Safety and Health Act of 1970 established the General Duty Clause, which required that employers to "furnish to each of his employees employment and a place of employment which are free from recognized hazards that are causing or likely to cause death or serious physical harm to his employees."[2827] Because of the broad all-hazards and all-workplaces language of the Act, OSHA can incorporate guidelines provided by other agencies, such as the CDC and NIH, effectively making compliance with the guidance mandatory.[2828]

HHS has integrated applicable laws, regulations, and best practices into the comprehensive guidance document titled, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*.[2829] BMBL guidance is broadly considered "the consensus code of practice for identifying and controlling biohazards," and adherence to the minimum requirements stated within the BMBL is enforced by regulators and all research institutions.[2830]

16.14.1.4 Grants and Contracts

Guidance documents can also be enforced by making adherence to a contractual requirement or a term and condition of award. NIH has employed this approach. NIH awardees are required, either through the terms and conditions of an awarded grant or contractually, to meet worker health and safety standards.[2831] US-based institutions receiving NIH funding for any recombinant or synthetic nucleic acid research must conduct biosafety risk assessment and risk management per the relevant *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*.[2832] Similarly, US-based institutions

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2825 Jennifer Gaudioso, Susan A. Caskey, LouAnn Burnett, Erik Heegaard, Jeffery Owens, Philippe Stroot, "Strengthening Risk Governance in Bioscience Laboratories," Sandia National Laboratories, SAND2009-8070, December 2009, p.37, http://www.biosecurity.sandia.gov/BioRAM/Biorisk%20Framework%20Report.pdf.

2826 For example: American Society of Heating, Refrigerating and Air-Conditioning Engineers, *ASHRAE Laboratory Design Guide*, 1st edition (2002).

2827 Occupational Safety and Health Administration (OSHA), "Laboratory Safety Guidance," OSHA 3404-11R, 2011, p.5, https://www.osha.gov/Publications/laboratory/OSHA3404laboratory-safety-guidance.pdf.

2828 Ibid.

2829 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*.

2830 National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version* (Washington: The National Academies Press, 2011), p. 79.

2831 National Institutes of Health, *NIH Grants Policy Statement* "4.1.12 Health and Safety Regulations and Guidelines," October 2013, http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch4.htm#health_safety_regulations.

2832 All NIH-funded projects using recombinant or synthetic nucleic acids and all projects at institutions that receive any NIH funding, must conform to these guidelines.
National Institutes of Health, "NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)," November 2013, <http://osp.od.nih.gov/sites/default/files/NIH_Guidelines_0.pdf>.

receiving any federal funding for life science research are required to conduct dual use review for experiments with certain agents and toxins, including HPAI and reconstructed 1918 influenza.[2833]

In general, the US National Institutes of Health provides funding to researchers whose institutions comply with applicable US requirements per the grant award or contractual agreement, and the researcher's country, "providing the foreign requirements do not contradict US laws."[2834] This includes compliance with Select Agent Regulations, human subjects' protections, animal care and use, recombinant DNA guidelines, and other requirements as applicable. That said, an assessment of the landscape of security governance and implementation at institutions outside the United States is extremely complex because laws for securing pathogens differs significantly among countries. In addition, different countries may categorize influenza, SARS-CoV, and MERS-CoV differently than the United States, which results in different applicable country-specific laws and practices associated with research with these viruses. Therefore, such an assessment would need to be country-specific and involve all relevant country stakeholders (including law enforcement or security entities) to better understand the legal and practical security environment in which US-sponsored research is conducted.

16.14.1.5 International Obligations

Some federal statutes and regulations serve to implement obligations derived from international agreements reached by the United States. For example, the US has implemented its commitments under the Biological Weapons Convention through legislation prohibiting biological weapons.[2835,2836] Additionally, US implementation of United Nations Security Council Resolution 1540 includes a variety of legislative acts, executive orders, and regulations.[2837,2838] Many international agreements require this corresponding implementation to become practically enforceable in the US; without thoughtfully crafted implementing statutes and regulation, enforcing international commitments is difficult.[2839,2840]

Development, production, stockpiling, and use of biological information or material for biological weapons purposes is outlawed by international law and is inconsistent with established international norms. The 1925 Geneva Protocol bans the use of bacteriological and asphyxiating agents in war.[2841] The 1972 Biological Weapons Convention (BWC) in essence bans the development, production, and

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2833 United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. (2014) Accessible at http://phe.gov/s3/dualuse/documents/durc-policy.pdf. Accessed on September 8, 2015.

2834 NIH National Institute of Allergy and Infectious Diseases, "NIAID Select Agent Policy for Foreign Institutions Questions and Answers," May 13, 2015, http://www.niaid.nih.gov/researchfunding/qa/pages/selagentfor.aspx#standard. Accessed November 11, 2015.

2835 Initial U.S. implementation was under the "Biological Weapons Anti-Terrorism Act of 1990" and has been updated under the "Anti-Terrorism and Effective Death Penalty Act of 1996" and the "USA Patriot Act" of 2001.
U.S. Code, Title 18 Chapter 10-Biological Weapons Section 175, "Prohibitions with respect to biological weapons" http://www.gpo.gov/fdsys/pkg/USCODE-2013-title18/pdf/USCODE-2013-title18-partI-chap10-sec175.pdf;

2836 Text of the Biological Weapons Convention, 1972 http://www.state.gov/t/isn/bw/c48738.htm.

2837 United Nations Security Council, *Resolution 1540* (2004) http://www.un.org/en/ga/search/view_doc.asp?symbol=S/RES/1540%20(2004);

2838 Highlights include the Public Health Security and Bioterrorism Preparedness Response Act of 2002 modifying 18 USC 2283, the National Defense Authorization Act of 1995 (Public Law 103-337) and the Federal Select Agent Program. A complete description of U.S. efforts under UNSCR 1540 can be found in October 11, 2013 letter from the U.S. to the U.N. http://www.un.org/en/ga/search/view_doc.asp?symbol=S/AC.44/2013/17.

2839 U.S. Supreme Court, *Medellin v. Texas*, 552 U.S. 491 (2008) (No. 06-984).

2840 U.S. Supreme Court, *Bond v. United States*, 564 U.S. ___ (2014) (No. 12-158).

2841 Note that several countries at the time made treaty reservations reserving the right to retaliate in kind and/or limiting the ban to cover only fellow Contracting Parties.
United Nations Office for Disarmament Affairs, "1925 Geneva Protocol: Protocol on the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare," http://www.un.org/disarmament/WMD/Bio/1925GenevaProtocol.shtml.

stockpiling of biological weapons.[2842] Most states have signed and ratified the treaty: the Convention has 173 States Parties and nine signatories.[2843] Only 14 UN-recognized states have not signed the Convention; of these, only Israel and Angola have substantial armed forces.[2844] The near-universality of the BWC means that a strong case can now be made that a norm against the development, production, and stockpiling of a biological weapon exists as a legally binding norm under international customary law.[2845] However, the Convention provides no mechanism for verification or enforcement, and some countries may be willing to flout their obligations, as was done for instance by the Soviet Union.[2846]

International organizations, like the World Health Organization, may also issue guidance that complements domestically-issued guidance, such as the *Laboratory Biosafety Manual* that complements the BMBL.[2847]

16.14.1.6 Practice

Safety and security at high containment facilities are shared responsibilities among many stakeholders, including the institution, Institutional Biosafety Committee, Institutional Review Entity, biosafety officer, principal investigator, researchers, support staff, and law enforcement. Professional societies like the American Biological Safety Association hold conferences that build a community of practice. Factors such as the safety and security culture and personal relationships with emergency response personnel drastically improve defenses but would not be captured by regulatory analysis.[2848]

In addition, institutions may implement measures beyond regulatory requirements. For instance, an institution can decide to treat certain pathogens as if they were Tier 1 Select Agents for the purposes of improved safety and security, going beyond what is required, or broadly recommended in authoritative guidance. Other institutions may implement additional physical security measures in order to safeguard their personnel and laboratory space.

That said, implementation of biosafety and biosecurity measures varies across research institutions. On one end of the spectrum are institutions that do not adequately comply with federal requirements and lose funding or approval to conduct certain research. On the other end of the spectrum are institutions that go well-above the minimum requirements for security as described in the governing documents. Therefore, generalization of implementation across all research institutions is not appropriate and was not done in this assessment.

2842 Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction, http://www.unog.ch/80256EDD006B8954/%28httpAssets%29/C4048678A93B6934C1257188004848D0/$file/BWC-text-English.pdf.

2843 United Nations Office at Geneva, The Biological Weapons Convention Implementation Support Unit, "Membership of the Biological Weapons Convention," http://www.unog.ch/__80256ee600585943.nsf/%28httpPages%29/7be6cbbea0477b52c12571860035fd5c?OpenDocument&ExpandSection=1#_Section1.

2844 The current list is: Angola, Chad, Comoros, Djibouti, Eritrea, Guinea, Israel, Kiribati, Micronesia (Federated States of), Namibia, Niue, Samoa, South Sudan, Tuvalu.

2845 Nicholas A. Sims, "Legal Constraints on Biological Weapons," *Deadly Cultures: Biological Weapons since 1945*, eds. Mark Wheelis, Lajos Rózsa, Malcolm Dando (Cambridge: Harvard University Press, 2006), p. 331.

2846 For a comprehensive history of the Soviet biological weapons program, see: Leitenberg M, Zilinskas R, (2012) *The Soviet Biological Weapons Program: A History* Cambridge: Harvard University Press.

2847 World Health Organization, *Laboratory Biosafety Manual – Third Edition* http://www.who.int/entity/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1.

2848 "Safety culture" as advocated by ABSA is also common in aviation and health care industries. Felix Gmuender and Daniel Fischer, *ABSA Conference Denver 2010*, "Assessing Safety Culture in Biorisk Facilities" http://www.absaconference.org/pdf53/Session12-Gmuender.pdf.

Because no systematic evaluation of all research institutions was possible for this assessment, measures implemented in practice reflect those in use at the research institutions project staff visited during the course of this assessment, which represent a total of six institutions conducting research involving influenza, SARS-CoV, and/or MERS-CoV. Five institutions are subject to the US Government's pause in funding and NIH's "stop work" order of GoF research. One institution that also has received a "stop work" order does not conduct any research with Biological Select Agents and Toxins.

16.14.2 Laws, International Agreements, and Guidance Documents

The following tabular list of laws, international agreements (including treaties and other international obligations), and guidance documents on biosafety and biosecurity was compiled as part of the above analysis on the policies and practices governing US laboratories in the biosafety and biosecurity spheres. The table provides the relevant item name as well as a hyperlink to allow retrieval of the item. For each item, the table contains a short summary highlighting the relevant aspects of the item for the current report. Each item is assigned a subjective relevance score to indicate how applicable the item was to the assessment in the current report (low relevance; 5 high relevance). Where applicable, the item is classed as "safety," "security," or "safety and security" -oriented, depending on the motivation behind the item. Finally, each item is given a topic classification based on the safety/security functions the item performs. The numbers are as follows:

1 Personnel surety
2 Physical/electronic access control
3 Inventory/accountability
4 Storage
5 Transfer, shipment, chain-of-custody
6 Surveillance and monitoring
7 Malicious actor detection
8 Incident Reporting
9 Emergency Response
0 Research Plan
A Waste disposal

Items having several different functions are given a combined number, in order. So for instance an item like 29 CFR 1910.1201 that deals with Inventory/accountability issues and transfer, shipment, and chain-of-custody issues, is assigned the number 35.

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
5 CFR 730-799	Federal Regulations		Security	5	0	Export Administration Regulations (Parts 730 to 780) and Additional Protocol Regulations (Part 781 to 799). The Commerce Control List under 5 CFR 738 regulates *inter alia* exports of pathogens by potentially requiring an export license depending on the pathogen and its destination (essentially for national security / global security reasons).	Link
7 CFR 330: Code of Federal Regulations, Title 7 "Agriculture," Part 330 "General Provisions"	Federal Regulations				0	Regulations on plant pests	Link
7 CFR 331: Code of Federal Regulations, Title 7 "Agriculture," Part 331 "Possession, Use, and Transfer of Select Agents and Toxins"	Federal Regulations				0	**[PPQ SELECT AGENTS]** Implementation of the Agricultural Bioterrorism Protection Act of 2002 (alongside 9 CFR 121). Note that the safety and security regulations under 7 CFR 331 are functionally equivalent to those laid out for USDA Select Agents and CDC Select Agents, albeit for different pathogens.	Link
7 CFR 331.3: Code of Federal Regulations, Title 7 "Agriculture," Part 331.3 "PPQ select agents and toxins"	Federal Regulations				0	**[PPQ SELECT AGENTS]** Lists plant pathogens classed as PPQ Select Agents and regulated by 7 CFR 331. PPQ is the "Plant Protection and Quarantine Programs of the Animal and Plant Health Inspection Service."	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
9 CFR 121: Code of Federal Regulations, Title 9 "Animals and Animal Products," Part 121 "Possession, Use, and Transfer of Select Agents and Toxins"	Federal Regulations					**[USDA SELECT AGENTS]** Implementation of the Agricultural Bioterrorism Protection Act of 2002 (alongside 7 CFR 331). The safety and security regulations under 9 CFR 121 are functionally equivalent to those laid out for PPQ Select Agents and CDC Select Agents, albeit for different pathogens. Influenza is a Veterinary Services Select Agent (VS Select Agents; i.e., USDA Select Agents).	Link
9 CFR 122: Code of Federal Regulations, Title 9 "Animals and Animal Products," Part 122 "Organisms and Vectors"	Federal Regulations					A permit issued by the USDA Secretary is required to transport any organisms or vectors across state/territory/district of Columbia lines or to import them into the United States, unless a permit has already been granted or the organism was produced at an establishment licensed under 9 CFR 102. The rest of 9 CFR 122 covers the permit application process and the suspension or revocation of permits process.	Link
9 CFR 161	Federal Regulations				2	Requirements and standards for Accredited Veterinarians.	Link
15 CFR Parts 730-774: Code of Federal Regulations, Title 15 "Commerce and Foreign Trade," Parts 730-774	Federal Regulations				0	Commerce and foreign trade regulations. Regulations 15 CFR 710 to 721 implement the Chemical Weapons Convention (CWC), see entry below. Regulation 15 CFR 744.6 calls for a Bureau of Industry and Security (BIS) license to export or transfer any item that could be used in development of a biological weapon.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
21 CFR 58: Code of Federal Regulations, Title 21 "Food and Drugs," Part 58 "Good Laboratory Practice for Nonclinical Laboratory Studies"	Federal Regulations			None	0	The set of regulations under 21 CFR 58 cover laboratory practices for nonclinical studies, but apply to testing facilities that do safety tests on test articles, and not clinical studies or field trials in animals. Therefore, its relevance for biological research laboratories considered in the current study is low. Two parts (58.81 and 58.90) are flagged here for comparative purposes. Part 58.81 "Standard Operating Procedures" sets the requirements for a standard operating plan, which must list instructions for a large number of common laboratory tasks (detailed in the code). For instance, instructions on how to conduct animal room preparation; on how to ensure animal care; on the "placement, transfer, and identification of animals"; on how to handle animals "found moribund or dead during a study"; and on the "maintenance and calibration of equipment." Animal care regulations are themselves detailed in Part 58.90 "Animal Care." These regulations include the isolation and health assessment of newly received animals, and the suitable identification of warm-blooded animals that are not suckling rodents that must be manipulated for "an extended period of time" or that must be removed from and returned to their cages for any reason (including cleaning).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
29 CFR 1910.38: Code of Federal Regulations, Title 29 "Labor," Part 1910.38 "Emergency action plans"	Federal Regulations	7 November 2002			3	The labor regulations under 29 CFR 1910 apply to "workplaces in general industry" apart from mobile workplaces (vehicles, vessels). 29 CFR 1910.38 specify regulations on emergency action plans required by OSHA. Such emergency action plans must include procedures: for reporting emergencies (such as fires); for emergency evacuation; for employees who must remain "to operate critical plant operations before they evacuate"; for employees performing rescue or medical duties; for ensuring all employees are accounted for after an evacuation; and contact information for employees that can be reached by other employees for information on the emergency action plan. There must be an alarm system to warn employees, and employees must be trained in assisting "in a safe and orderly evacuation of other employees."	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
29 CFR 1910.120: Code of Federal Regulations, Title 29 "Labor," Part 1910.120 "Hazardous waste operations and emergency response"	Federal Regulations				4	The hazardous waste operations and emergency response regulations under 29 CFR 1910.120 explicitly apply to biological agent hazards, as a "hazardous substance" includes "any biological agent and other disease-causing agent which after release into the environment" may cause adverse effects in individuals (including "death, disease, behavioral abnormalities"). The regulation stipulates that employees must have a written safety and health plan for employees for normal facility work with hazardous wastes, and a site-specific safety and health plan for such tasks. Mandated elements of a site-specific plan of particular relevance to this report include the requirement for written "lines of authority, responsibility, and communication" the provision of personal protective equipment (PPEs) and when necessary decontamination showers, and the setup of a medical surveillance program. The facility must have developed and communicated decontamination procedures to employees. The facility must also have an emergency response plan which must include *inter alia*: emergency alerting procedures, the provision of PPEs and emergency equipment, the provision of emergency medical treatment and first aid, and specific decontamination procedures that are not covered by the safety and health plan. This emergency response plan must be "rehearsed regularly."	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
29 CFR parts 1910.132-138, Annex A and Annex B: Code of Federal Regulations, Title 29 "Food and Drugs," Part 1910.132-138, Annex A and Annex B "Subpart I- Personal Protective Equipment	Federal Regulations			1	1	The regulations set requirements for selecting, providing, maintaining, and replacing personal protective equipment.	Link
29 CFR 1910.1030: Code of Federal Regulations, Title 29 "Labor," Part 1910.1030 "Blood borne pathogens"	Federal Regulations	Last amended 3 April 2012; initial 6 December 1991			5	These regulations apply to all occupational exposure to "human blood, human blood components, and products made from human blood," to "pathogenic microorganisms that are present in human blood and can cause disease in humans" (such as Hepatitis B virus and human immunodeficiency virus), and a defined list of human bodily fluids, unfixed human tissue or organs, and "HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV."	Link
29 CFR 1910.1200: Code of Federal Regulations, Title 29 "Labor," Part 1910.1200 "Hazard communication"	Federal Regulations	Last amended 8 February 2013; initial 9 February 1994			0	These regulations on hazard communications explicitly do not apply to "biological hazards" as per 6). xii).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
29 CFR 1910.1201: Code of Federal Regulations, Title 29 "Labor," Part 1910.1201 "Retention of DOT markings, placards and labels"	Federal Regulations	19 July 1994		35	1	Relevant portion: any individual receiving a hazardous material shipment that must be marked, labelled, or placarded, must retain the "markings, labels and placards" required under US Department of Transportation's Hazardous Materials Regulations (49 CFR 171 through 180). "For non-bulk packages which will not be reshipped, the provisions of this section are met if a label or other acceptable marking is affixed in accordance with the Hazard Communication Standard (29 CFR 1910.1200)."	Link
29 CFR 1910.1450: Code of Federal Regulations, Title 29 "Labor," Part 1910.1450 "Occupational exposure to hazardous chemicals in laboratories"	Federal Regulations	Last amended 22 January 2013; initial 31 January 1990			2	The regulations promulgated under this part regard occupational chemical exposure hazards. However, the part includes a section "I. Laboratory Security" which is also applicable for biosecurity assessments.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
29 CFR 1926: Code of Federal Regulations, Title 29 "Labor," Part 1926 "Safety and Health Regulations for Construction"	Federal Regulations				0	Establishes regulations regarding safety of employees during construction that are extremely similar to those under 29 CFR 1910. Of note, 1926.65 "Hazardous Waste Operations and Emergency Response" establishes regulations for "emergency response operations for releases of, or substantial threats of releases of, hazardous substances without regard to the location of the hazard." A hazardous substance is defined as a "substance which, by reason of being explosive, flammable, poisonous, corrosive, oxidizing, irritating, or otherwise harmful, [are] likely to cause death or injury."	Link
39 CFR 20: Code of Federal Regulations, Title 39 "Postal Service," Part 20 "International Postal Service"	Federal Regulations			5	2	International mail manual. The international mail manual itself has regulations (Section 601.10.17) on transporting infectious substances through USPS.	Link
40 CFR parts 150-189: Code of Federal Regulations, Title 40 "Protection of the Environment," Parts 150-189 "Subchapter E- Pesticide Programs"	Federal Regulations			None	0	Regulations on pesticides. 40 CFR 160 establishes regulations for "good laboratory practice standards" for conducting studies "that support or are intended to support applications for research or marketing permits for pesticide products regulated by the EPA."	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 71.54: Code of Federal Regulations, Title 42 "Public Health," Part 54 "Import regulations for infectious biological agents, infectious substances, and vectors"	Federal Regulations	4 February 2013		5	2	Regulations governing the importation of "infectious biological agents, infectious substances, and vectors" into the US from abroad. Such activities are prohibited without a permit. The CDC issues permits which then detail the specific requirements and conditions placed on the sample (which can include restrictions on intra-state transfer once in the US). The importer must implement "biosafety measures commensurate with the hazard posed by the infectious biological agent, infectious substance, and/or vector to be imported, and the level of risk given its intended risk." The importer must also "help ensure" that the shipper complies with all applicable legal requirements "concerning the packaging, labeling, and shipment of infectious substances."	Link
42 CFR 72 [RESERVED]	Federal Regulations			None	0	**Not valid anymore [Reserved]**	
42 CFR 73.0: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 0 "Applicability and related requirements"	Federal Regulations	Amended 4 December 2012; original 5 October 2012		3	5	**[CDC SELECT AGENTS]** Possession of SARS-CoV, Lujo virus, Chapare virus must be reported to CDC on or before December 2012. Compliance with the rest of 42 CFR 73 is required for new registrees by April 3, 2013 and already registered possessors by December 4, 2012.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
42 CFR 73.3: Code of Federal Regulations. Title 42 "Public Health," Part 73 "Select Agents", subsection 3 "HHS select agents and toxins"	Federal Regulations	Last amended 12 May 2014; original 18 March 2005			5	**[CDC SELECT AGENTS]** Defines the CDC's HHS select agents and toxins, and identifies certain of these select agents and toxins as Tier 1 select agents and toxins. SARS-CoV and "Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)" are both select agents, but are *not* Tier 1 select agents. MERS-CoV is as of August 2015 *not* a select agent.	Link
42 CFR 73.4: Code of Federal Regulations. Title 42 "Public Health," Part 73 "Select Agents", subsection 4 "Overlap select agents and toxins"	Federal Regulations	Last amended 12 May 2014; original 18 March 2005			1	**[CDC SELECT AGENTS]** Defines overlap select agents and toxins, and identifies certain of these overlap select agents and toxins as Tier 1 overlap select agents and toxins. Overlap agents and toxins are those subject to regulation by both CDC and APHIS. SARS-CoV, MERS-CoV, and influenza are as of August 2015 *not overlap* select agents and toxins.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.7: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 7 "Registration and related security risk assessments"	Federal Regulations			35	5	**[CDC SELECT AGENTS]** Possession, use, or transfer of HHS select agent or toxin requires a certificate of registration issued by the HHS Secretary (exceptions exist as listed in 73.5 for clinical and diagnostic labs shipping select agent pathogens/toxins in specimens for diagnosis or verification or for proficiency testing, as well as for products given specific exemptions. These are irrelevant here.) The Attorney General must do a risk assessment before granting registration and the HHS Secretary needs to base the decision to grant registration on this assessment. This assessment is based on information provided by those seeking registration through APHIS/CDC Form 1, and can also be based on inspection or submission of additional documents prepared under 42 CFR 73 requirements (such as the security plan). Certificate of registration is valid for a maximum of 3 years. **[CDC SELECT AGENTS]** Those registering need to designate a Responsible Official.	Link
42 CFR 73.8: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 8 "Denial, revocation, or suspension of registration"	Federal Regulations			1A	1	**[CDC SELECT AGENTS]** Provides clauses for denying, revoking, or suspending a certification of registration. If a certification of registration is revoked or suspended, all work with select agents and toxins must stop. The select agents and toxins must be safeguarded, and if HHS requests it they must be disposed of as requested.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.9: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 9 "Responsible Official"	Federal Regulations	Amended 5 October 2012; original 18 March 2005		3	3	**[CDC SELECT AGENTS]** Sets requirements and duties of the Responsible Official. They must carry out an annual documented inspections of registered laboratories that stored or used pathogens. They must also report the identification of select agents and toxins contained in diagnosis or verification specimens within seven calendar days after identification for SARS-CoV and reconstructed influenza virus in diagnosis or verification specimens and within 90 days for proficiency testing specimens; the reporting is done through APHIS/CDC Form 4 and a copy of the form must be kept for three years (clauses are more stringent for some pathogens; requires telephone call reporting).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.10: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 10 "Restricting access to select agents and toxins; security risk assessments"	Federal Regulations	Amended 5 October 2012; original 18 March 2005	Safety & Security	1	5	**[CDC SELECT AGENTS]** An individual's access to a select agent or toxin must be pre-approved by the HHS Secretary or HHS Administrator, following a security risk assessment conducted by the Attorney General. Access is defined as the possession of a select agent or toxin (such as the ability to use, manipulate, carry) or the ability to gain possession of a select agent or toxin. The approval is valid for a maximum of three years. The individual must have "the appropriate education, training, and/or experience to handle or use such agents or toxins." The regulation provides clauses so that HHS can deny, limit, or revoke an individual's access approval for safety or security reasons. Further, should an individual's access to select agents or toxins be terminated by their entity (not HHS), the Responsible Official must "immediately notify" CDC or APHIS and must present the reason(s) behind the decision (for instance, a researcher changing laboratories).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.11: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 11 "Security"	Federal Regulations	Last amended 12 May 2014; original 18 March 2005	Security	123456789	5	**[CDC SELECT AGENTS]** A security plan must be developed and implemented by those registering to meet CFR 73 regulations that is "sufficient to safeguard the select agent or toxin against unauthorized access, theft, loss, or release." The security plan must address 10 specific security-related topics (see link), which can be summarized as covering procedures for routine operations (cleaning, maintenance, repairs), for facility security (such as establishing a minimum of three security barriers, setting reporting requirements, implementing inventory control, securing storage of select agents and toxins, following cyber-security measures, and inspecting suspicious packages outside of areas where select agents and toxins are used or stored, to prompt first response of security forces), for transfers of select agents or toxins (shipping to another entity, intra-entity transfers), for emergency response (removing unauthorized or suspicious personnel, responding to exposure of animals or plants, to address security compromises such as lost keys, to communicate with law enforcement), and to provide for personnel training and personnel protocols (reporting channels).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.12: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 12 "Biosafety"	Federal Regulations	Amended 5 October 2012; original 18 March 2005	Safety	09	5	**[CDC SELECT AGENTS]** A biosafety plan must be developed and implemented by those registering to meet CFR 73 regulations. It must include descriptions of the biosafety and containment procedures for the select agent or toxin as well as "any animals (including anthropods) or plants intentionally or accidentally exposed to or infected with a select agent."	Link
42 CFR 73.13: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 13 "Restricted experiments"	Federal Regulations	Last amended 12 May 2014; original 18 March 2005	Safety & Security	0	5	**[CDC SELECT AGENTS]** Restricts the conduct of certain experiments and the possession of results from said restricted experiments, unless approved and conducted as requested by the HHS Secretary. The experiments restricted are those that: "involve the deliberate transfer of, or selection for, a drug resistance trait to select agents that are not known to acquire the trait naturally, if such acquisition could compromise the control of disease agents in humans, veterinary medicine, or agriculture" or "experiments involving the deliberate formation of synthetic or recombinant DNA containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD[50] <100".	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.14: Code of Federal Regulations. Title 42 "Public Health," Part 73 "Select Agents", subsection 14 "Incident response"	Federal Regulations	Amended 5 October 2012; original 18 March 2005	Safety & Security		5	**[CDC SELECT AGENTS]** A written incident response plan must be developed based on a site-specific risk assessment. This plan must be kept available for review by "employees" (no further explanation), and the incident response plan must be exercised at least yearly. There are specific additional requirements for facilities with Tier 1 select agents. The incident response plan must, *inter alia*, "fully describe the entity's response procedures for the theft, loss, or release of a select agent or toxin; inventory discrepancies; security breaches (including information systems); severe weather and other natural disasters; workplace violence; bomb threats and suspicious packages; and emergencies such as fire, gas leak, explosion, power outage, and other natural and man-made events."	Link
42 CFR 73.15: Code of Federal Regulations. Title 42 "Public Health," Part 73 "Select Agents", subsection 15 "Training"	Federal Regulations	5 October 2012	Safety & Security	58	5	**[CDC SELECT AGENTS]** Those registering to meet CFR 73 regulations must provide training on biosafety, security, and incident response for personnel to be working with select agents or toxins, or that will enter areas where select agents or toxins are stored or handled. The training must be done before that person is granted access by HHS. Refresher training must be done annually, or whenever there is a "significant" amendment to biosafety, security, or incident response plans. Training must be logged. Facilities holding Tier 1 select agents must in addition conduct yearly specific annual insider threat awareness training.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.16: Code of Federal Regulations. Title 42 "Public Health," Part 73 "Select Agents", subsection 16 "Transfers"	Federal Regulations		Safety & Security	5	3	**[CDC SELECT AGENTS]** Regulations for the transfer of select agents or toxins, which can only be conducted between individuals or entities registered to possess select agents or toxins. CDC or APHIS approval is required before a transfer unless the Select Agent is contained in a specimen for proficiency testing, in which case CDC or APHIS must simply be informed at least 7 days prior to the transfer (unless the transferors are both under the same entity for the registration). Authorization for transfer is sought by submitting APHIS/CDC Form 2. If the select agent or toxin has not been received within 48 hours after the slated delivery date, or if the package is damaged "to the extent that a release of the select agent or toxin may have occurred," the receiver must immediately notify CDC or APHIS.	Link
42 CFR 73.17: Code of Federal Regulations. Title 42 "Public Health," Part 73 "Select Agents", subsection 17 "Records"	Federal Regulations		Safety & Security	34	5	**[CDC SELECT AGENTS]** Records of the name and characteristics ("strain, GenBank Accession number, etc."), the quantity acquired and date and source of acquisition, the storage location, the movement in-and-out of storage of the sample and the individual(s) who moved the sample, intra-entity transfer records, external transfer records, and a list of all animals and plants intentionally or accidentally exposed to or infected with a select agent must be kept for any select agent "held in long-term storage." Similar regulations are established for toxins (omitted here).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
42 CFR 73.18: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents", subsection 18 "Inspections"	Federal Regulations			3	1	**[CDC SELECT AGENTS]** Allows the HHS secretary to inspect without prior notification any site where activities regulated by 42 CFR 73 take place, and will be allowed to inspect and copy relevant records. The HHS secretary can conduct an inspection prior to issuing a certificate of registration (As also noted in CFR 73.7).	Link
42 CFR 73.19: Code of Federal Regulations, Title 42 "Public Health," Part 73 "Select Agents," subsection 19 "Notification of theft, loss, or release"	Federal Regulations		Safety & Security	89	5	**[CDC SELECT AGENTS]** Requires immediately reporting the theft or loss of a select agent or toxin to CDC or APHIS and to "appropriate Federal, State, or local law enforcement agencies," without exception (for instance regardless of whether the select agent or toxin is then identified, or the responsible parties found). A completed APHIS/CDC Form 3 must then be submitted within seven calendar days. Also requires immediately reporting the" release of an agent or toxin causing occupational exposure or release of a select agent or toxin outside of the primary barriers of the biocontainment area" to CDC or APHIS. A completed APHIS/CDC Form 3 must then be submitted within seven calendar days.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
49 CFR 171.15 and 171.16: Code of Federal Regulations, Title 49 "Transportation," Part 171.15 "Immediate notice of certain hazardous materials incidents," and Part 171.16 "Detailed hazardous materials incident reports"	Federal Regulations	Last amended 20 July 2011; initial 3 December 2003	Safety	59	2	171.15 requires individuals with physical possession of a hazardous material to notify by telephone the National Response Center "as soon as is practical but no later than 12 hours after" when certain types of incidents involving the hazardous material occurs. This specifically includes "fire, breakage, spillage, or suspected contamination" involving an infectious substance other than regulated medical waste. They are then also required to fill out a detailed incident report (Hazardous Materials Incident Report on DOT Form F 5800.1 (01/2004)) within 30 days of the incident. The report parameters are detailed in 171.16.	Link
49 CFR 172.802: Code of Federal Regulations, Title 49 "Transportation," Part 802 "Components of a security plan"	Federal Regulations	16 April 2008	Security	5	0	Requires a security plan for transportation of certain hazardous materials, but Division 6.2 materials (infectious substances) are not one of the listed hazardous materials covered by this set of regulations.	Link

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Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
49 CFR 173.134: Code of Federal Regulations, Title 49 "Transportation," Part 173.134 "Class 6, Division 6.2- Definitions and exceptions"	Federal Regulations	Last amended 11 March 2013; initial 14 August 2002		5	2	49 CFR Parts 171 to 180 regulate the transport of hazardous materials. Under 49 CFR 173.134, infectious substances are called "Division 6.2" materials, and are defined as materials "known or reasonably expected to contain a pathogen" (except neutralized or inactivated materials). Infectious substances are then categorized as either Category A or Category B. Category A is for an "infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs", whereas Category B is for an infectious substance that is not in such a form. Classification of an infectious substance as Category A or B "must be based on the known medical history or symptoms of the source patient or animal, endemic local conditions, or professional judgment concerning the individual circumstances of the source human or animal."	Link
49 CFR 173.196: Code of Federal Regulations, Title 49 "Transportation," Part 173.196 "Category A Infectious substances"	Federal Regulations	Last amended 7 January 2013; initial 14 August 2002	Safety	5	2	Regulations for the shipment of Category A substances are given under 49 CFR 173.196. The triple-packing requirement is detailed. The primary receptacle must be capable of resisting given pressure and temperature ranges without leaking. See link for details.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
49 CFR 173.199: Code of Federal Regulations, Title 49 "Transportation," Part 173.199 "Category B Infectious substances"	Federal Regulations	Last amended 8 January 2015; initial 14 August 2002	Safety	5	2	Regulations for the shipment of Category B substances are given under 49 CFR 173.199. Category B substances must be triple-packed (two receptacles and a rigid outer packaging), and the requirements for each layer of packaging are laid out (see link). In case of transportation by aircraft, the package is inspected for leakage; if leakage is detected, then the cargo compartment must be disinfected. The regulation has a training component requiring that "each person who offers or transports" a Category B infectious substance know of the requirements under this regulation section.	Link
49 CFR 178.609: Code of Federal Regulations, Title 49 "Transportation," Part 178.609 "Test equipment for packagings for infectious substances"	Federal Regulations	Last amended 7 September 2004; initial 21 December 1990	Safety		1	Provides regulations on the test standards for packaging materials required for infectious substances (and hence, for Category A and Category B agents).	Link
"Occupational Safety and Health Act"	Federal Laws	Last amended 6 October 1992; initial 29 December 1970	Safety		1	This law empowered the Secretary of Labor to enact regulations on occupational safety of employees engaged in hazardous waste operations.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
"Public Health Security and Bioterrorism Preparedness and Response Act of 2002"	Federal Laws	12 June 2002	Safety and Security		5	The part most relevant for this report is Title II, "Enhancing Controls on Dangerous Biological Agents and Toxins." The sections under this Title amended the Antiterrorism and Effective Death Penalty Act of 1996 controls on biological agents (see below). In particular, the act adds text to require the Secretary to enact regulation that became the Select Agents regulations, i.e., on possession (and on barring possession from restricted persons), transfers, and incident reporting for dangerous pathogens per the dangerous pathogens list (the Select Agents, although not called as such in the act).	Link
... "Agricultural Bioterrorism Protection Act of 2002," within the "Public Health Security and Bioterrorism Preparedness and Response Act of 2002"						Title II of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, "Enhancing Controls of Dangerous Biological Agents and Toxins," has a Subtitle B cited as the "Agricultural Bioterrorism Protection Act of 2002." This text created the Secretary of Agriculture's Select Agents list and associated regulations for pathogens with "the potential to pose a severe threat to animal or plant health, or to animal or plant products."	
"US Patriot Act"	Federal Laws	26 October 2001	Security		1	Updates 18 USC 175 (see below) under Section 817, notably by adding a definition of "restricted persons" and making it so that such persons are prohibited from having access to Select Agents.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
18 USC 175: "Prohibitions with Respect to Biological Weapons"	Federal Laws	Last amended 12 June 2002; initial 22 May 1990	Security		1	Codifies the BWC's Articles I and III into US national law by criminalizing the "development, production, transfer, acquisition, retention, or possession of any biological agent, toxin, or delivery system for other than prophylactic, protective, bona fide research, or other peaceful purposes." The law's official short-hand is the 'Biological Weapons Anti-Terrorism Act of 1989'.	Link
Antiterrorism and Effective Death Penalty Act of 1996	Federal Laws	24 April 1996	Security		3	Contains text on enhanced control over biological agents, as well as enhanced penalties for unauthorized possession of biological agents. Creates the first Select Agents list (although not called as such in the act) by requiring "the Secretary [...to] establish and maintain a list of each biological agent that has the potential to pose a severe threat to public health and safety."	Link
Executive Order 13546, "Optimizing the Security of Biological Select Agents and Toxins in the United States"	Executive Order	2 July 2010	Safety and Security		5	Established the risk-based tiering of the Select Agents, into Select Agents and Tier 1 Select Agents.	Link
Executive Order 13486, "Strengthening Laboratory Biosecurity in the United States"	Executive Order	9 January 2009	Security		0	Created a Working Group on Strengthening the Biosecurity of the United States within the Department of Defense with a mandate to review the effectiveness of relevant laws, regulations, guidance, and practices.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
Biological Weapons Convention	International Treaty	Signed 10 April 1972; entered into force 26 March 1975	Security		1	The Biological Weapons Convention's Article III reads: "Each State Party to this Convention undertakes not to transfer to any recipient whatsoever, directly or indirectly, and not in any way assist, encourage, or induce any State, group of States or international organizations to manufacture or otherwise acquire any of the agents, toxins, weapons, equipment or means of delivery specified in Article I of the Convention." The treaty text does not contain steps that State Parties must take to be in compliance with this Article. In practice, States Parties such as the US have passed national laws and established regulations ("National Implementation") that restrict access to dangerous pathogens and criminalize unauthorized access. More specifically, the US Biological Weapons Anti-Terrorism Act of 1989 (enacted 1990, amended 1996) provided for the BWC's implementing (see its entry above).	Link
Chemical Weapons Convention	International Treaty	Signed 13, 1993; entered into force 29 April 1997	Security		0	The Chemical Weapons Convention (CWC) regulates *inter alia* toxin production and stockpiling. The US has established a series of regulations under 15 CFR 710 to 721 for the national implementation of the CWC.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
Australia Group	Informal international grouping		Security		0	The Australia Group is an informal group of states (including the US) that seek to harmonize export controls on chemical and biological agents and equipment. The Australia Group maintains Common Control Lists that are meant as guides of what to restrict through state-level national export control laws and regulations. The Common Control List regarding Human and Animal Pathogens and Toxins for Export Control includes "SARS-CoV-related coronavirus," "Avian influenza viruses of high pathogenicity" (as defined by WHO, the EU, or competent national regulatory bodies) and "Reconstructed 1918 influenza virus," as well as certain genetic elements thereof.	Link
UNSCR 1540	Legally-binding UNSCR	April 2004	Security		0	United Nations Security Council Resolution (UNSCR) 1540 is a legally-binding resolution on all UN Member States that requires these states to deploy measures against biological, chemical, and nuclear weapons proliferation, "including appropriate laws and regulations to control export, transit, trans-shipment and re-export." Of relevance here is the establishment of dangerous pathogen export controls prompted/assisted by the 1540 Committee's work.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
World Health Assembly Resolution 58.29 (2005)	Int'l Agreement	25 May 2005	Safety		1	Urges member states to take a number of measures under the "Enhancement of Laboratory Biosafety" rubric, including to: review lab safety protocols; implement programs to promote biosafety for safe handling and transport; develop national preparedness plans and plans to enhance lab compliance with biosafety guidelines for lab practices; and to facilitate international access to lab biosafety equipment (such as PPEs).	Link
International Health Regulations, World Health Assembly Resolution 58.3 (2005)	Int'l Agreement	May 2005	Safety		0	IHR is legally binding for all WHO member states, since WHA 58.3 is a World Health Assembly resolution that adopts the IHR. The regulations are "to prevent, protect against, control and provide a public health response to the international spread of disease."	Link
OECD Best Practice Guidelines for Biological Resource Centers	Int'l Agreement	March 2007				The Organisation for Economic Co-operation and Development (OECD) has issued the "OECD Best Practice Guidelines for BRCs [Biological Resource Centers]." OECD member countries (including the US) agreed to these guidelines in March 2007.	Link
Army Regulations 50-1	Military regulations	28 July 2008	Safety & Security		3	Army Regulations regarding biosafety/biosecurity. Chapter 2 details the personnel reliability program process to be followed. Chapter 3 details pathogen and toxin control and inventory management. Chapter 4 details Army procedures for transport. Chapter 5 details the occupational health program process to be followed. Chapter 6 details the security program process to be followed. Chapter 7 lays out the incident response process. Chapter 8 details the surety program evaluations procedures.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
Security Guidance for Select Agent or Toxin Facilities	Guidance	5 July 2013	Security		5	42 CFR 73.11 notes that those designing a security plan "should consider" this document. The document provides guidance on how to implement the required security aspects of the Select Agents regulations.	Link
Guidance on the Inventory of Select Agents and Toxins	Guidance	Last revised 16 April 2015; initial 12 October 2012	Safety and Security	34	5	Guidance on proper storage and inventory management for select agents.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules	Guidance	Amended 6 November 2013; Initial 24 June 1994			5	NIH Guidelines on the conduct of recombinant or synthetic nucleic acid molecule research. All NIH funded projects on the topic, as well as non-NIH funded projects on the topic carried out at or supported by institutions that receive NIH funding, must conform to these guidelines (p.11). Influenza generated by recombinant or synthetic methods are to be run under the biosafety level that would be used if dealing with the virus from which the majority source of segments came from (p.21). BSL-3 enhanced containment is to be used for all influenza viruses "containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1)," apart from in a few select cases as detailed in Sections III-D-7-a and –b where containment can be brought down to BL-2 (p.21-22). Both SARS -CoV and MERS-CoV are classed as Risk Group 3 pathogens (high individual risk, low community risk) on a 1 to 4 scale (with 4 being high individual risk and high community risk pathogens).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
CDC Public Health Guidance for Community-Level Preparedness and Response to Severe Acute Respiratory Syndrome (SARS-COV), Supplement F "Laboratory Guidance"	Guidance	3 May 2005	Safety		1	Supplement F of the CDC's 2005 guidance document on SARS-CoV provides laboratory biosafety guidelines for working with specimens associated with SARS-CoV. The document focuses on preparedness for a potential public health response (diagnostics, specimens potentially containing SARS-CoV)	Link
Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition	Guidance	Amended December 2009; initial 1984	Safety & Security	12345678 90A	5	42 CFR 73.12 notes that those designing a biosafety plan "should consider" this document. NIH and CDC-led "national code for biosafety." A thorough set of best practices for biosafety risk assessment and implementation. Includes a chapter on biosecurity. Chapters are broken down into separate entries in this dataset due to the large scope and relevance of the content.	Link
... "Section I - Introduction"			Safety & Security	12345678 90A	5	Provides an overview of the principles of biosafety and biosecurity, relevant to the rest of the BMBL guidance. While the substance is not in this section, it outlines the ways to think about biosafety and how security fits into that context	
... "Section II - Biological Risk Assessment"			Safety & Security	12345678 90A	5	Overview of the factors to consider and the methods to use when determining the risk of work with a given infectious agent. Includes 2 primary categories: agent hazards and procedure hazards.	

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
… "Section III - Principles of Biosafety"			Safety & Security	123456A	5	Overview of the principles to biosafety, factors involved in choosing a BSL, import/shipment, select agents. This chapter points to other chapters and appendices for more detail. While not explicitly about biosecurity, these concepts overlap with security measures	
…"Section IV - Laboratory Biosafety Level Criteria"			Safety & Security	12346A	5	Specifications for BSL 1-4 lab safety, including standard microbiological practices, special practices, safety equipment (primary barriers/PPE), and laboratory facilities requirements for each BSL level.	
…"Section V - Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities"				12346A	5	Specifications for ABSL 1-4 lab safety, including standard microbiological practices, special practices, safety equipment (primary barriers/PPE), and laboratory facilities requirements for each ABSL level.	
… "Section VI - Principles of Laboratory Biosecurity"			Safety & Security	12345689	5	Considerations for planning and implementing a biosecurity program (examples, not standards). Relationship between security measures and safety measures.	
…"Section VII - Occupational Health and Immunoprophylaxis"			Safety	16	5	Best practices for detection and mitigation of laboratory-acquired infections (LAIs), may include medical exams, vaccines, reporting procedures, testing for exposed employees, and treatment plans	

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
... "Section VIII - Agent Summary Statements"			Safety & Security	125689	5	Background, laboratory and natural modes of transmission, and laboratory safety and containment recommendations for a large variety of pathogens and toxins. This are not comprehensive guidance but starting points for safety and security planning. Recommends BSL levels and additional special measures for research with many different pathogens.	
... "Appendix A - Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets"			Safety	16A	5	Detailed information about how to set up a biosafety cabinet	
... "Appendix B - Decontamination and Disinfection"			Safety	16A	5	Disinfection and sterilization procedures, planning, and characterization	
... "Appendix C - Transportation of Infectious Substances"			Safety & Security	5	5	Shipping, transport, and transfer codes and some summary guidance	
... "Appendix D - Agriculture Pathogen Biosafety"			Safety & Security	12346A	5	Requirements for BSL-3-Ag, and BSL-3, Enhanced. Requires BSL-3-Ag containment for all work with HPAI	
... "Appendix E - Arthropod Containment Guidelines (ACG)"			Safety	0	0	References to the Arthropod Containment Levels and guidelines developed by the American Committee of Medical Entomology	

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
... "Appendix F - Select Agents and Toxins"			Safety & Security	123456789	5	References applicable Select Agents and Toxins codes, summarizes those codes.	
... "Appendix G - Integrated Pest Management (IPM)"			Safety	2689	2	Pest control specifications and guidance	
... "Appendix H - Working with Human, NHP and Other Mammalian Cells and Tissues"			Safety	1260A	1	Describes requirements and recommendations for working with human/primate/mammalian cells, including OSHA regulations, recommended prophylactic vaccinations, and recommended handling practices/risk assessments.	
... "Appendix I - Guidelines for Work with Toxins of Biological Origin"			Safety & Security	124689	1	Recommendations for training, facilities planning, safety equipment, handling aerosols/spills/sharps incidents, safety precautions and waste disposal/decontamination.	
... "Appendix J - NIH Oversight of Research Involving Recombinant Biosafety Issues"			Safety	0	5	Introduction to the NIH rDNA guidelines and the IBC/RAC processes.	
Public Health Service and NIH Office of Laboratory Animal Welfare "Policy on Humane Care and Use of Laboratory Animals"	Policy	Revised 2015			0	Policy ensuring animal welfare	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
International Guiding Principles for Biomedical Research Involving Animals	Guidance	December 2012			0	International guidance on ensuring laboratory animal welfare prepared by the Council for International Organization of Medical Sciences (CIMS) and the International Council for Laboratory Animal Science (ICLAS)	Link
Guide for the Care and Use of Laboratory Animals, Eighth Edition	Guidance	2011			0	Guidance on ensuring laboratory animal welfare and ethical use of animals, prepared by the National Research Council of the National Academies' Committee for the Update of the Guide for the Care and Use of Laboratory Animals.	Link
ASHRAE laboratory design guide, 1st edition	Guidance		Safety		3	Technical book on laboratory design	Book
WHO Laboratory Safety Manual, 3rd Edition	Guidance	2004; initial 1984	Safety		5	A WHO publication that provides guidance on topics including: laboratory biosafety, codes of practice in laboratories, laboratory equipment operation, good microbiology techniques, contingency and emergency planning, disinfection and sterilization, transport of infectious substances, biosafety considerations for recombinant DNA technology, hazardous chemicals, fire and electrical safety, the concept of a biosafety officer and a biosafety committee, ensuring the safety of support (repair, cleaning) staff, and safety checklists. The manual also has two pages on biosecurity.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
WHO Biorisk Management: Biosecurity Guidance		September 2006	Security		3	WHO guidance for all member states on addressing biosecurity issues. The proposed approach is to start by identifying valuable (and/or particularly dangerous) biological material that needs to be safeguarded. The document confirms that "there is no international agreement on what kind of biosafety containment level and laboratory biosecurity practices should apply for specific situations" (p.21).	Link
ABSA biosecurity task force white paper: understanding biosecurity	Guidance	January 2003	Security		1	A one-page document on biosecurity	Link
CEN Workshop Agreement, CWA 15793 "Laboratory biorisk management standard"	Guidance	February 2008	Safety and Security		1	The European Committee for Standardization (CEN) convened a workshop, "CEN Workshop 31-Laboratory biosafety and biosecurity," which resulted in this agreement. The agreement covers both biosafety and biosecurity risks. The United States is not a CEN member, but there was US participation at the workshop (both direct and through a public comment process).	Link
US Department of Transportation. "Transporting Infectious Substances Safely"	Guidance		Safety	5	1	This document helps practitioners comply with US Department of Transportation regulations on hazardous material transportation (49 CFR 171 to 180).	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity

Item	Item Type	Date	Safety/ Security ?	Safety/ Security Functions	Subjective Relevance (1=low 5=high)	Summary	Link
IATA Dangerous Goods Regulations	Guidance		Safety	5	1	The Dangerous Goods Regulations, 56th edition, is prepared by the International Air Transport Association (IATA). It includes guidance for air transport of infectious substances, but the guidance document is not freely available and must be purchased.	No free copy.
IATA Guidance Document: Infectious Substances	Guidance	Now out of date; 2010	Safety	5	1	A brief and now-out-of-date guidance document regarding air transport of infectious substances. Dedicates one page (p.5) to spill mitigation procedures and first aid. The document is valid for the Dangerous Goods Regulations 52nd edition from 2010, whilst the current regulations are on their 56th edition. IATA also offers a training course on the topic, but it is not free (http://www.iata.org/training/courses/Pages/infectious-substances-tcgp43.aspx)	Link
WHO Guidance on regulations for the Transport of Infectious Substances 2015-2016	Guidance	Applicable 1 January 2015, covers 2015-2016	Safety	5	1	WHO has published guidance on regulations (i.e., model regulation) on the transport of infectious substances every two years, based on the broader recommendations established as the "United Nations Recommendations on the Transport of Dangerous Goods" program. The document includes specific guidance for topics such as: shipping medical waste and infected animals; transport by air, rail, road, sea, and post; and spill emergency response regulations.	Link

Table 16.5. Laws, Regulations, International Agreements, and Guidance Documents on Biosafety and Biosecurity							
Item	**Item Type**	**Date**	**Safety/ Security ?**	**Safety/ Security Functions**	**Subjective Relevance (1=low 5=high)**	**Summary**	**Link**
Strengthening Risk Governance in Bioscience Laboratories," Sandia National Laboratories. SAND2009-8070	Guidance	December 2009	Safety and Security			The document provides an overview of means to reduce biosafety and biosecurity risks, and in carrying out a thorough risk appraisal process. Appendix C describes the BioRAM Model, which is an algorithm to appraise the risk at the pathogen-specific level.	Link

16.15 Restriction of Fundamental Research, Dual Use Research of Concern and Recombinant DNA Guidelines

The current US Government's deliberative process and pause of certain GoF research, relates to years of discussion and policymaking for scientific research that could be used for beneficial or military/harmful purposes. Federal policies on the dual use of scientific research encompass the export control regime, communication of fundamental scientific research, recombinant DNA guidelines, and policies on oversight of dual use life sciences research. Export control requirements are incorporated in the detailed assessment of security measures in Appendix V: Section 16.11. Policies on communication of fundamental research, dual use life sciences research of concern, and recombinant DNA provide the overarching framework under which past and future life science research occurs. Because they are central to biosecurity considerations of GoF pathogens but are not physical or personnel security measures, these policies are briefly described below.

16.15.1 National Security Decision Directive 189

In 1982, President Reagan issued National Security Decision Directive (NSDD)-189, *National Policy on the Transfer of Scientific, Technology and Engineering Information,*[2849] which states:

- "that, to the maximum extent possible, the product of fundamental research remain unrestricted."
- "that, where the national security requires control, the mechanism for control of information generated during federally-funded fundamental research in science, technology and engineering at colleges, universities and laboratories is classification," and
- that "no restrictions may be placed upon the conduct or reporting of federally-funded fundamental research that has not received national security classification, except as provided in applicable US statutes."

In this policy, fundamental research is defined as "basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons." This policy repeatedly has been upheld since its issuance.

16.15.2 Dual Use Life Sciences Research Concern

The 2012 debate about publication of specific mutations in the H5 influenza gene that resulted in mammalian transmissible H5 influenza viruses catalyzed the issuance of the United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern[2850] in March 2012 and the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern in September 2014.[2851] These policies established requirements for review and oversight of life sciences

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[2849] President Ronald Reagan, National Security Decision Directive 189 – National Policy on the Transfer of Scientific, Technical and Engineering Information, September 21, 1985.

[2850] United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern, (2012) Accessible at http://www.phe.gov/s3/dualuse/documents/us-policy-durc-032812.pdf. Accessed on September 9, 2015.

[2851] United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern, (2014) Accessible at http://phe.gov/s3/dualuse/documents/durc-policy.pdf. Accessed on September 8, 2015.

research involving one of 15 agents (14 pathogens and one toxin), which includes highly pathogenic avian influenza virus and the reconstructed 1918 influenza virus, and one of seven categories of experiments that raise particular concern:

1. Enhancement of harmful consequences of certain agents or toxins,
2. Disruption of immunity or the effectiveness of an immunization against certain agents or toxins without clinical or agricultural justification,
3. Alteration of an agent or toxin to confer resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against the agent or toxin or to facilitate evasion of detection methodologies,
4. Increase in the stability, transmissibility, or ability to disseminate certain agents or toxins,
5. Alteration of the host range or tropism of certain agents or toxins,
6. Enhancement of susceptibility of a host population to certain agent or toxins, and
7. Generation or reconstitution of an eradicated or extinct agent or toxin.

US government agencies that fund life sciences research are required to develop agency-specific requirements to implement the Federal (March 2012) "dual use research of concern" (DURC) policy. The September 2014 institutional DURC oversight policy describes an organizational framework for oversight of research that has dual use potential and provides a list of responsibilities for the institution, principal investigator, and federal government. The National Institutes of Health provides a Companion Guide for the dual use policies, which includes identification and assessment of research, a framework for institutional review, development and review of a risk mitigation plan, and communication of research with dual use potential.[2852] In addition, the NIH provides a series of case studies to assist scientific organizations implement the policy.[2853] These case studies are intended to illustrate how to apply the policy to the review of life science research.

In practice, the Institutional Biosafety Committees of several academic and nonprofit research institutions review research for dual use potential.[2854] Some institutions have established specific committees who review research for its dual use potential.[2855] In 2012, some research institutions stopped reviewing research for its dual use potential because they no longer conduct select agent research.[2856] However, some of these institutions may resume reviewing research for dual use potential if they conduct research with any quantity of botulinum toxin, per the September 2014 institutional DURC oversight policy.[2857]

[2852] National Institutes of Health. Tools for Identification, Assessment, Management, and Responsible Communication of Dual Use Research of Concern. A Companion Guide to the United States Government Policies for Oversight of Life Sciences Dual Use Research of Concern. Sept 2014. Accessible at http://www.phe.gov/s3/dualuse/Documents/durc-companion-guide.pdf. Accessed on September 18, 2015.

[2853] National Institutes of Health. Implementation of the USG Policy for Institutional Oversight of Life Sciences DURC: Illustrative Case Studies. September 2014. Accessible at http://www.phe.gov/s3/dualuse/Documents/12-case-studies-durc.pdf. Accessed on September 18, 2015.

[2854] AAAS, AAU, APLU, FBI. Bridging Science and Security for Biological Research: A Discussion about Dual Use Review and Oversight at Research Institutions. Workshop Report. 2012. Accessible at http://www.aaas.org/report/discussion-about-dual-use-review-and-oversight-research-institutions. Accessed on September 18, 2015.

[2855] Ibid.

[2856] Ibid.

[2857] United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. (2014) Accessible at http://phe.gov/s3/dualuse/documents/durc-policy.pdf. Accessed on September 8, 2015.

16.15.2.1 HHS Framework for H5N1 and H7N9

In 2013, the US Department of Health and Human Services (HHS) issued its framework for guiding funding decisions on H5N1 and H7N9 GoF research, specifically that which involves transmission among mammals by respiratory droplets.[2858,2859,2860]

The H5N1/H7N9 framework builds on the existing funding agency "standard review" process for GoF research that increases aerosol transmission of the viruses. The standard review process involves an initial peer review for scientific merit and subsequent dual use review if the research meets the US government definition for "dual-use research of concern," as stipulated by the March 2012 DURC policy.[2861,2862,2863] Once this standard review process has been completed, projects "reasonably anticipated to generate an HPAI H5N1 virus that is transmissible between mammals by respiratory droplets" must meet the following seven criteria before it can be considered for funding by an HHS funding entity:

1. The resultant virus "could be produced through a natural evolutionary process."
2. The project would address "a scientific question with high significance to public health,"
3. "No feasible alternative methods [exist] to address the same scientific question in a manner that poses less risk" than the proposed project,
4. The potential biosafety risks "to laboratory workers and the public can be sufficiently mitigated and managed,"
5. The biosecurity risks "can be sufficiently mitigated and managed,"
6. The research is "anticipated to be broadly shared in order to realize its potential benefits to global health," and
7. The research "will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research."[2864]

If a project meets all seven criteria as determined by the HHS funding entity, it enters a HHS department-level review to determine whether the proposal is acceptable for HHS funding based on the following considerations:[2865] A) the quality of the risk assessments; B) additional factors that may affect the decision; C) required risk mitigation measures; and D) the project's place within the broader HHS

2858 A Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets, p. 4, https://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf.

2859 Harold Jaffe, Amy P. Patterson, Nicole Lurie, "Extra Oversight for H7N9 Experiments," appeared in *Science (Letters)* 341, no. 6147 (7 August 2013): p.713-714, http://www.sciencemag.org/content/341/6147/713.2.full.

2860 Harold Jaffe, Amy P. Patterson, Nicole Lurie, "Extra Oversight for H7N9 Experiments," Nature (Correspondence) 500, no. 151 (8 August 2013), http://www.nature.com/nature/journal/v500/n7461/full/500151a.html.

2861 Amy P. Patterson et al., "A Framework for Decisions About Research with HPAI H5N1 Viruses," *Science (Policy Forum)* 339, no. 6123 (1 March 2013): p. 1037, http://www.sciencemag.org/content/339/6123/1036.

2862 United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern, p.1-2, http://www.phe.gov/s3/dualuse/Documents/us-policy-durc-032812.pdf.

2863 United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern. (2012) Accessible at http://www.phe.gov/s3/dualuse/documents/us-policy-durc-032812.pdf. Accessed on September 9, 2015.

2864 A Framework for Guiding U.S. Department of Health and Human Services Funding Decisions about Research Proposals with the Potential for Generating Highly Pathogenic Avian Influenza H5N1 Viruses that are Transmissible among Mammals by Respiratory Droplets, p. 4.

2865 Ibid.

H5N1/H7N9 influenza portfolio.[2866] If the departmental review results in a positive determination, the project may be funded.[2867] For all HHS-funded H5N1/H7N9 projects, researchers must report to HHS "any unanticipated results that involve the generation of a virus that is transmissible among mammals by respiratory droplets."[2868]

16.15.3 NIH Guidelines for Research Involving Recombinant DNA and Synthetic Nucleic Acid Molecules

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules describes safety practices and containment procedures for life sciences research involving recombinant or synthetic nucleic acid molecules. Synthetic nucleic acid molecules were added to the Guidelines in 2013.[2869] The purpose of the Guidelines is "to specify the practices for constructing and handing: (i) recombinant nucleic acid molecules, (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and (iii) cells, organisms, and viruses containing such molecules."[2870] The NIH Guidelines are contractually required for any institution receiving support from the National Institutes of Health. Other US government agencies also require compliance with the NIH Guidelines for receipt of life science grants involving recombinant DNA.[2871,2872]

The NIH Guidelines require research institutions to assess and categorize the risk of research involving recombinant or synthetic nucleic acid molecules by Risk Group, which are defined in the Guidelines.[2873] They provide details about:

- The level of containment of research based on the experiments involved to prevent environmental release of microorganisms, plants, or animals that contain recombinant or synthetic nucleic acid molecules.
- Requirements for Institutional Biosafety Committees (IBCs) to review, approval and oversight of research involving recombinant or synthetic nucleic acid molecules.
- Composition of the IBC,
- Experiments covered by the NIH Guidelines, including

2866 Ibid.
2867 Ibid.
2868 Ibid.
2869 National Institutes of Health. Frequently Asked Questions: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Accessible at http://osp.od.nih.gov/sites/default/files/Synthetic_FAQs_April_2013.pdf. Accessed on September 18, 2015.
2870 National Institutes of Health. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Nov 2013. Accessible at http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html#_Toc351276217. Accessed on September 18, 2015.
2871 Department of Defense. General Guidelines for Awards Funded by the Department of Defense (DoD). Accessible at http://www.usamraa.army.mil/pages/pdf/General_Guidelines_for_Awards_Funded_by_the_DoD.pdf. Accessed on September 18, 2015.
2872 National Institutes of Health. Frequently Asked Questions: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Accessible at http://osp.od.nih.gov/sites/default/files/Synthetic_FAQs_April_2013.pdf. Accessed on September 18, 2015.
Although no reference is includes, other U.S. Departments and Agencies, such as the Department of Homeland Security, includes compliance with the NIH Guidelines as a requirement for receiving research funding.
2873 National Institutes of Health. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Nov 2013. Accessible at http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html#_Toc351276217. Accessed on September 18, 2015.

  - Experiments that must be reviewed by IBCs, such as experiments involving transfer of drug resistance traits into microorganisms and cloning of genes for toxin molecules,
  - Experiments that require IBC and Institutional Review Board (for human subjects research) approval,
  - Experiments that require IBC approval before initiation for each Risk Group,
  - Experiments involving infectious DNA or RNA viruses or defective DNA and RNA viruses in the presence of helper viruses,
  - Experiments involving animals or plants, and
  - Experiments involving influenza viruses,
- Experiments that are exempt from the NIH Guidelines,
- Roles and Responsibilities of the research institution, principal investigator, and NIH,
- Information to be submitted to the NIH,
- Major and minor actions, and
- Responsibilities and composition of the Recombinant DNA Advisory Committee (RAC), who provide advice on matters concerning research that involves recombinant or synthetic nucleic acids, and may review and approve certain experiments.

The NIH provides information about major actions taken and experiments that are exempt from the NIH Guidelines.[2874]

All academic, non-profit, and for-profit research institutions receiving NIH or other US government research funding are required to have an IBC that is registered with the NIH. Although the guidance is voluntary, several companies that do not receive federal funding have also established an IBC that is registered with the NIH.

16.16 Analysis of Security Measures: Requirements, Implementation, and Gaps of Security Measures

Security measures reviewed for this assessment fall into seven categories: training; personnel reliability; physical security; surveillance and monitoring; storage, inventory, and accountability processes; transfer, shipment, and chain-of-custody protocols; and emergency response. In this section, we define each of these categories and describe requirements and implementation practices in non-Select Agent, Select Agent, and Tier 1 Select Agent operating environments.

[2874] National Institutes of Health. NIH Guidelines website. Accessible at http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines. Accessed on September 18, 2015.

16.16.1 Training

Training is required to ensure that all workers understand the risks associated with their research, and appropriate measures to address those risks.

16.16.1.1 General – At All Levels

Requirements

General US labor laws enforced by OSHA require all biological researchers to received basic safety training. In addition, OSHA requires employees and students working in research laboratories receive training on exposure to hazardous chemicals, hazard communication, blood borne pathogens, personal protective equipment, eye and face protection, hand protection, and respiratory protection.[2875]

The Biosafety in Microbiological and Biomedical Laboratories describe training associated with different biosafety level laboratories.[2876] The primary goal of training personnel in appropriate laboratory safety procedures, including preventing, detecting, and reporting unsafe behavior, is to reduce the risk of accidental exposure. Typical biosafety training includes practical procedures for working in the laboratory, donning required personal protective equipment, signage indicating the hazards in the laboratory and emergency contacts, reporting procedures in case of laboratory accidents or negligence, and decontamination measures in case of an accident. In addition, scientists should receive training to demonstrate technical proficiency at the appropriate biosafety level and to demonstrate knowledge about hazards of specific infectious agents.

In addition, scientists conducting federally-funded research with any quantity of botulinum toxin that involves at least one of the seven categories of experiments, and is considered to have dual use potential are required to receive DURC training by their institutions.[2877]

Implementation at Research Institutions

Research institutions provide training to employees and staff on basic laboratory safety, materials safety, blood borne pathogens, hazard waste disposal, use of sharps, and information security. Several institutions provide training on dual use research of concern and recombinant DNA guidelines.

Researchers working in high containment laboratories receive agent-specific training, which includes understanding clinical symptoms, several weeks of hands-on mentored training, and knowledge of standard operating procedures. Many laboratories conduct hands-on, mentored training in stages, allowing new recruits to proceed to higher containment levels (BSL-3) only after they have demonstrated competence at a lower containment level (BSL-2) or same containment level (BSL-3). This accompanied training process ensures that each individual demonstrates proficiency and competency in conducting experiments safely and according to standard operating procedures. In addition, researchers are informed of facility security and access, visitor access, entry requirements, and facility-specific policies in addition to training about waste management, emergency response, and use of sharps in high containment.[2878]

[2875] Occupational Safety and Health Administration. Laboratory Safety Guidance. OSHA 3404-11R 2011. Accessible at https://www.osha.gov/Publications/laboratory/OSHA3404laboratory-safety-guidance.pdf. Accessed on September 18, 2015.

[2876] Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. 2009. Accessible at http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf. Accessed on September 18, 2015.

[2877] United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. (2014) Accessible at http://phe.gov/s3/dualuse/documents/durc-policy.pdf. Accessed on September 8, 2015.

[2878] Lesley C. Homer et al., "Guidelines for Biosafety Training Programs for Workers Assigned to BSL-3 Research Laboratories," Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science 11, no. 1 (2013): p.13.

Laboratories working with animals tend to have a heightened awareness of the potential for animal rights extremists trying to gain access to research facilities or recruiting scientists to assist their efforts.

16.16.1.2 Additional Measures at the Select Agent level

Requirements

In addition to the training requirements for all laboratories and high-containment laboratories, the BSAT regulations specifically require employee security awareness training. Each entity that is registered to possess Select Agents must have a security plan.[2879] The security plan must have provisions to ensure "that all individuals with access approval [to a Select Agent] understand and comply with the security procedures" described in the plan.[2880] The entity must implement these measures by providing information and training on security topics, such as security awareness, to any individual with approval and access to BSAT facilities.[2881] Training for employees with Select Agent access must be conducted at least once a year and a written record must be kept that details the training, including "the means used to verify that the employee understood the training."[2882]

In addition, scientists conducting federally-funded research with at least one of the 15 agents that involves at least one of the seven categories of experiments and is considered to have dual use potential are required to receive DURC training by their institutions.[2883]

Implementation at Research Institutions

Researchers approved to work with BSAT receive hands-on, mentored training similar to that described for research in high containment laboratories. Some of the BSAT laboratory personnel interviewed described training routines that went well beyond the minimum required to meet regulatory requirements. Trainers and mentors test the knowledge gained by researchers to assess the degree to which they understand the standard operating procedures and laboratory safety and security practices.

Research institutions provide training to BSAT approved staff about security considerations associated with working in BSAT laboratories. One institution offers insider threat training provided for Tier 1 BSAT researchers to non-Tier 1 BSAT researchers; the non-Tier 1 BSAT researchers also attend this training. Another institution provides training on security risks and updates this training as information on threats presents itself.

Institutions train scientists through laboratory drills and exercises, which is described in the Emergency Response section. Research institutions are encouraged to train individuals on defining and responding to "suspicious activity" and appropriate responses to security emergencies.[2884]

2879 U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security," <http://www.ecfr.gov/cgi-bin/text-idx?SID=94bd3a730b8387eb15bc058bc4637627&mc=true&node=se42.1.73_111&rgn=div8>.

2880 U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

2881 42 CFR 73.15. U.S. Government Publishing Office, "Title 42: Public Health, §73.15 Training," <http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=0f9c35f1c983d1c04a020889c033b02b&mc=true&n=pt42.1.73&r=PART&ty=HTML#se42.1.73_115>.

2882 42 CFR 73.15. U.S. Government Publishing Office, "Title 42: Public Health, §73.15 Training."

2883 United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. (2014) Accessible at http://phe.gov/s3/dualuse/documents/durc-policy.pdf. Accessed on September 8, 2015.

2884 LouAnn C. Burnett, "Biosafety Practices Associated with Potential Agents of Biocrime and Biowarfare," Emerging Technologies, Supplement 3, 1A.2.5.

16.16.1.3 Additional Measures at the Tier 1 Select Agent Level

Requirements

Institutions are required to provide insider threat training to Tier 1 BSAT researchers. Because insider threat trainings are only required for personnel working with Tier 1 BSAT, it not mandatory for the pathogens considered in this report under current regulations.[2885]

Implementation at Research Institutions

Several institutions that support Tier 1 BSAT research provide insider threat training to appropriate researchers. One institution has their local FBI Weapons of Mass Destruction Coordinator conduct the training. This institution offers the training to its non-Tier BSAT researchers, several of whom attend voluntarily. Other institutions provide their own insider threat training.

16.16.1.4 Gap Analysis

Based on the above information, the following gaps were identified:

- The ability of training provided to inculcate security awareness at non-BSAT facilities, particularly at facilities that do not work with animals, is unclear. Security recommendations provided in guidance documents at the non-BSAT level contain little guidance to enhance security awareness among employees and staff.[2886] Current text in authoritative guidance documents, including first and foremost the BMBL, is written to assist laboratory managers in implementing a security plan, but provides little to no advice for the average laboratory workers to become more security-conscious.[2887] and

- At all levels, the ability to maintain high-quality training depends on laboratory personnel resources and funding. BSAT approved research institutions do not receive additional financial support to pay for additional staff dedicated to training BSAT researchers.

16.16.2 Personnel Reliability

Personnel reliability measures seek to prevent insider threats through initial vetting and periodic monitoring of employees and students with access to BSAT. This vetting process involves both background checks and related measures and reliability assessments, which include demonstration of competency and proficiency in high containment laboratories. Similar demonstrations of competency and proficiency in high containment laboratories are conducted in laboratories that are not regulated by the BSAT Regulations.[2888]

[2885] This is specified under 73.15b). U.S. Government Publishing Office. "Title 42: Public Health, 73.15 Training." <http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=0f9c35f1c983d1e04a020889c033b02b&mc=true&n=pt42.1.73&r=PART&ty=HTML#se42.1.73_115>.

[2886] In a 2003-2004 survey of Select Agent researchers conducted by Sandia National Laboratories, 53% of respondents state that their facilities provide biology-specific security training, versus 26.5% who said they did not. 73.5% of respondents further stated that the security training was done in conjunction with biosafety training. Sandia National Laboratories, "Laboratory Biosecurity: A Survey of the U.S. Bioscience Community," SAND No. 2006-1197P, Unlimited Release, February 2006, p. 6, http://www.biosecurity.sandia.gov/ibtr/subpages/pdfs/surveyResponses022606.pdf.

[2887] U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p.104-113.

[2888] AAAS. Biological Safety Training Programs as a Component of Personnel Reliability. Workshop Report. 2009. Accessible at http://www.aaas.org/sites/default/files/AAAS-Biosafety-report.pdf. Accessed on September 18, 2015.

16.16.2.1 General– At All Levels

Requirements

In non-security intensive environments, personnel reliability measures serve three purposes: 1) to ensure that only personnel who are able to work competently and reliably under high containment conductions have access to the laboratories; 2) to identify and address potential issues that may increase an individual's propensity to make mistakes or act negligently in the laboratory; and 3) identify and appropriately address export control requirements.

The description about competency and proficiency training in the previous section inform personnel reliability measures in high containment laboratories.[2889]

Deemed exports refers to the release of technology subject to the Export Administration Requirements (EAR) for biological research to a foreign national who is not a permanent resident or protected individual.[2890] This Export Administration Regulation (EAR) applies to pathogens restricted by the Australia Group and Select Agents.[2891] All research in the United States is subject to EAR, except if the technology is part of fundamental research, publicly available, or has been or will be published among other exceptions. With respect to biological research, most research conducted at university laboratories are not subject to EAR because it is considered fundamental research (the definition of which is the same as in NSDD-189). If the research is not considered fundamental because restrictions have been applied (e.g., restrictions on publication and proprietary information), it is subject to deemed export regulations. If the research involves controlled pathogens, a determination of the conductions for information sharing with a foreign national must be undertaken. Thus, the need for deemed export licenses appears to depend not on the pathogen per se, but the conditions associated with the research, such as a restricted publication.

In addition, the International Traffic in Arms Regulations require foreign nationals would need a permit to access any biological agents "modified to increase…capability to produce casualties in humans or livestock."[2892]

Implementation at Research Institutions

As described in the previous Training section, several research institutions provide hands-on, mentored training to researchers to ensure they demonstrate competency and proficiency of standard operating procedures and biosafety. In addition, several research institutions promote an opt-out policy to encourage researchers to voluntarily remove themselves from the laboratory if they are experiencing personal issues, such as illness, exhaustion, or personal distractions.[2893] These researchers are not punished; they are able to return to laboratory work once the distraction has been resolved.

2889 Ibid.

2890 Department of Commerce. Deemed Exports and Fundamental Research for Biological Items. Accessible at https://www.bis.doc.gov/index.php/policy-guidance/product-guidance/chemical-and-biological-controls/14-policy-guidance/deemed-exports/111-deemed-export-and-fundamental-research-for-biological-items. Accessed on September 18, 2015.

2891 U.S. Department of Commerce, *Commerce Control List*, "Category 1 – Special Materials and Related Equipment, Chemicals, 'Microorganisms' and 'Toxins'," <http://www.bis.doc.gov/index.php/forms-documents/doc_download/989-ccl1>.

2892 22 CFR 121.1(XIV)(b) U.S. Government Publishing Office, "The United States Munitions List," <http://www.ecfr.gov/cgi-bin/text-idx?SID=88e7fab9254a3319c3df6fb11a2233ab&mc=true&node=se22.1.121_11&rgn=div8>.

2893 AAAS. Biological Safety Training Programs as a Component of Personnel Reliability. Workshop Report. 2009. Accessible at http://www.aaas.org/sites/default/files/AAAS-Biosafety-report.pdf. Accessed on September 18, 2015.

Research institutions have dedicated offices to comply with export control requirements.[2894] In addition, some larger institutions designate an "Export Controls Coordinator" to help laboratories comply with "deemed export" regulations.[2895]

16.16.2.2 Additional Measures at the Select Agent level

Requirements

The BSAT Regulations require Security Risk Assessments (SRA) for all individuals seeking access to BSAT.[2896] SRA are required before initial approval and every three years. The assessment focuses on denying access to individuals known or suspected of having committed a serious crime, use illegal drugs, adjudicated as a mental defective, are a national of a country or acts on behalf of a country that has "repeatedly provided support for acts of international terrorism" as determined by the Secretary of State, or are themselves involved with terrorists or organized criminals. The exact criteria can be found in the HHS Select Agents regulations, more specifically under Title 42 "Public Health," Code of Federal Regulations (CFR) 73.10 "Restricting access to Select Agents and Toxins; security risk assessments."[2897] SRAs are conducted by the FBI Criminal Justice Information Services, who conduct a series of database checks to assess whether applicants should be granted access to BSAT laboratories.[2898]

The BSAT Regulations require all individuals with access to Select Agents and Toxins to report suspicious behavior or signs or evidence of a physical security or inventory accounting compromise, which then enables the responsible official to respond and revoke access as necessary.[2899] The regulations also require that the laboratory have a reporting process in place so that employees know how to report suspicious activity, and so that the responsible official knows how to pass on reports to the appropriate law enforcement agencies as necessary.[2900]

The institution's Responsible Official can suspend or revoke an individual's access to Select Agents and Toxins if necessary.[2901]

In addition to the SRA required by the BSAT Regulations, Army Regulation 50-1 details requirements for biosurety that apply to all individuals who work with DoD materials.[2902]

2894 A simple internet search identifies institutional information about export control regulations at a number of research institutions.

2895 For example: http://www.colorado.edu/vcr/export-controls/guidance/biological-agents

2896 U.S. Government Publishing Office, "Title 42: Public Health, §73.10 Restricting Access to select agents and toxins; security risk assessments," <http://www.ecfr.gov/cgi-bin/text-idx?SID=5e7f78178a77b6cce9961262ade5aa4&mc=true&node=se42.1.73_110&rgn=div8>.

2897 U.S. Government Publishing Office, "Title 42: Public Health, §73.10 Restricting Access to select agents and toxins; security risk assessments."

2898 The candidate provides fingerprints and a completed FD-961 form. NSABB, "Guidance for Enhancing Personnel Reliability and Strengthening the Culture of Responsibility: A Report of the National Science Advisory Board for Biosecurity," p. 17.

2899 As per the reporting requirements under 42 CFR 73.11(7)(i)-(v). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

2900 42 CFR 74.11(6)-(8). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

2901 NSABB, "Guidance for Enhancing Personnel Reliability and Strengthening the Culture of Responsibility: A Report of the National Science Advisory Board for Biosecurity," p. 17.

2902 Headquarters of the Department of the Army, "Army Regulation 50-1: Biological Surety," Nuclear and Chemical Weapons and Materiel, Unclassified, p. 10-20, <http://www.apd.army.mil/pdffiles/r50_1.pdf>.

Implementation at Research Institutions

Despite the costs involved, several institutions conduct background checks of all employees as part of the hiring practice. A few institutions are required to have personnel undergo several different types of national and international personnel security evaluations.

Institutions that support BSAT research require individuals seeking access to the BSAT to undergo the SRA process. Although these assessments occur every three years, the FBI reportedly performs additional spot checks by running names through up-to-date databases roughly every six months.[2903] The benefit of these checks relies on the types of databases used and the information contained therein.

Though not prevalent, a few research institutions conduct terrorism database checks, fingerprint employees, conduct a health assessment, and/or check international databases. In addition, some institutions have implemented an employee tracking system to determine personnel actions and who has or does not have access to facilities.

Good interactions with co-workers, support staff, administrators, and supervisors enhance personnel reliability measures, a comment echoed in the specialized literature as a requirement for an effective behavioral monitoring program.[2904]

Interviewees described the community of BSAT researchers, including those working with influenza and SARS-CoV, as close-knit. This environment promotes observation and timely reporting of behavior considered out-of-place or abnormal in the laboratory work space.

The self- and peer-reporting approach reduces insider threat risk and complements the required individual security risk assessment, although its effectiveness greatly relies on the reporting and security culture of a laboratory. The self- and peer-reporting approach will be ineffective in laboratories where workers and managers fear retaliation, wish to avoid additional paperwork, have overwhelming trust in their coworkers, or distrust their superiors. The effectiveness of the self- and peer-reporting approach also depends on the laboratory's leadership maintaining a close relationship with workers: "a leader who is engaged with his or her staff, who greets them by name and is perceived as accessible and caring, is more likely to be able to prevent an employee from becoming disgruntled, be aware of potential problems, and be better able to intervene to prevent the employee from becoming a crisis."[2905]

16.16.2.3 Additional Measures at the Tier 1 Select Agent level

Requirements

Personnel reliability measures for Tier 1 Select Agents involve a pre-access suitability assessment and a formal continuous suitability assessment process, in addition to the reliability measures established for

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2903 NSABB, "Guidance for Enhancing Personnel Reliability and Strengthening the Culture of Responsibility: A Report of the National Science Advisory Board for Biosecurity," p.17-18;
National Science Advisory Board for Biosecurity, "Enhancing Personnel Reliability among Individuals with Access to Select Agents," Report for the National Science Advisory Board for Biosecurity, May 2009, p. 12, <http://osp.od.nih.gov/sites/default/files/resources/NSABB%20Final%20Report%20on%20PR%205-29-09.pdf>.
As per 42 CFR 73.10(2)(j). U.S. Government Publishing Office, "Title 42: Public Health, §73.10 Restricting Access to select agents and toxins; security risk assessments."

2904 Ibid;
David R. Franz, Balancing Our Approach to the Insider Threat," Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science 9, no. 3 (2011): p.206.

2905 David R. Franz, Balancing Our Approach to the Insider Threat," Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science 9, no. 3 (2011), p.206.

Select Agents.[2906] Personnel reliability reporting requirements are stricter at the Tier 1 level. Regulations mandate the "self- and peer-reporting of incidents or conditions that could affect an individual's ability" to access, work with, or safeguard Select Agents.[2907]

Implementation at Research Institutions

Several institutions have established behavioral threat assessment teams to conduct the suitability assessment of researchers seeking or approved to work with BSAT.[2908] Other institutions have established occupational health programs where appropriately trained staff conduct periodic behavioral assessments of BSAT researchers.

16.16.2.4 Gap Analysis

Based on the above information, the following gaps were identified:

- Despite the requirement and implementation of personnel security efforts, a self-radicalized individual who has no criminal history and is careful not to communicate with extremists or other criminals would be extremely difficult to detect and, hence, unlikely to be screened out. Similarly, an individual with a pattern of threatening activities not reported to police, such as a propensity of becoming easily angered and agitated, will not be flagged by the SRA.[2909] Moreover, an insider has time to become radicalized, affiliated with a criminal organization, dependent on illegal drugs, or otherwise vulnerable or malicious in between personnel reliability checks.[2910] and

- Currently, institutions do not have a single system in which information about BSAT approved individuals can be stored and accessed by both police and research administers. Such a system would allow administrators to highlight potentially issues and police to determine whether any approved individual has gotten in trouble by the police.

16.16.3 Physical Security

Physical security measures are designed to prevent unauthorized access to the laboratory, in particular to protect pathogens and research animals. Examples of physical security measures include locks, physical barriers, security guards, restricted access policies, and a security guard.

16.16.3.1 General– At All levels

Requirements

Minimal access control measures are defined by the biosafety requirements (see Figure 16.4 above). Visitors to a laboratory at any level beyond BSL-1 must meet entry and exit requirements set by the facility managers. A lab at BSL-2 must have self-closing lockable doors, and a lab at BSL-3 and above must restrict access to the facility (i.e., through locked doors). US laboratory design standards incorporate

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[2906] 42 CFR 73.11 (f)(1),(3)(iii). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2907] 42 CFR 73.11 (f)(3)(i). Ibid.
[2908] AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Personnel Security Programs*, p. 13.
[2909] NSABB, "Guidance for Enhancing Personnel Reliability and Strengthening the Culture of Responsibility: A Report of the National Science Advisory Board for Biosecurity," p.17-18.
[2910] Initially, these checks were conducted once every five years, but these concerns led to the current 3-year regulation. See: NSABB, "Guidance for Enhancing Personnel Reliability and Strengthening the Culture of Responsibility: A Report of the National Science Advisory Board for Biosecurity," p. 16.

certain unnamed required security components as per the *NIH Design Requirements Manual for Biomedical Laboratories and Animal Research Facilities*.[2911] All "NIH owned and leased new buildings and renovated facilities" must comply with the NIH Design Requirements, and must therefore include these unnamed security features.[2912] These security features are detailed in a document, the *NIH Physical Security Design Requirements*, and is not to be released to the public.[2913]

As summarized in Figure 16.4 above, certain physical security measures are required for facilities housing animals. Research involving animals is conducted at an ABSL facility or at a BSL-3-Ag facility. Vivarium security is emphasized in guidance and facility design documents for such facilities, including the BMBL, in part as a result of the long history of incidents involving animal rights extremists. ABSL standards recommend that such facilities have no windows. This precaution imposes a barrier to entry by malicious actors by making them find out where the animals are stored and by preventing access through breaking of windows.[2914] If windows are nevertheless included in the facility design, the *NIH Design Requirements Manual for Biomedical Laboratories and Animal Research Facilities* stipulates that vivarium "windows must be designed to preclude the visualization of animals from outside of the building and also to address security issues."[2915] All facilities housing animals must also have self-closing doors. This feature defends against cases where a malicious actor would open animal cages in the hopes of causing an animal release.

The door lock type is important, as different lock types present different access control and access revocation benefits or challenges. Guidance in written documentation discourage the use of vulnerable traditional locks with regular keys (lock-and-key systems) because of the ease with which such locks can be picked, the necessity of physically retrieving keys from employees that are supposed to lose facility access, the ease with which the keys can be duplicated, and the lack of personnel tracking functionality given that all personnel keys are identical.[2916] High security cores provide stronger protection without introducing electronic vulnerabilities.[2917] Card, code, and biometric locks are more secure than traditional locks and typically also have logging capability, enabling security personnel to verify who accessed the laboratory at what time.[2918] These electronic systems are favored in laboratories that have the funds to incorporate them, as they facilitate billing users per hour of lab use and also double as a monitoring feature. Whether any US BSL-3 laboratories not working with Select Agents solely rely on weak lock-and-key systems is a knowledge gap. Although discouraged by all authors, lock-and-key systems are still listed as a security option in publicly-available literature on laboratory security design, mainly because it remains the least costly access control system.[2919]

2911 The National Institutes of Health, Division of Technical Resources, "Design Requirements Manual," p. 1-79, <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/Documents/Design%20Requirements%20Manual/NIH%20Design%20Requirements%20Manual%20ver%205-13.pdf>.

2912 Ibid.

2913 Ibid.

2914 Ibid.

2915 Ibid.

2916 National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version* (Washington: The National Academies Press, 2011), p. 257.

2917 Ibid.

2918 National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version*, p. 257. Ibid, p. 257.

2919 Mechanical key locks remain a listed lock type noted in Appendix III: "Comparison of Access Control Devices and Systems which are used to Control Access to Select Agents and Toxins", of: Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins, Animal and Plant Health Inspection Service (APHIS) Agriculture Select Agent Program. "Security Guidance for Select Agent or Toxin Facilities: 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73," p.33. See also: Daniel D. Watch, *Building Type Basics for Research Laboratories, second edition* (Hoboken: John Wiley & Sons, Inc., 2008), p.40.

Implementation at Research Institutions

In practice, BSL-3 laboratories have at least two physical barriers (and in many instances, several more "layered defenses") between an outsider and the laboratory space where pathogens are manipulated or stored. Often, but not always, different types of access controls are used to allow access to laboratories. These types of controls can be electronic, physical, or human.

The implementation of physical security measures at a facility has been reported in terms of the approximate time that a hypothetical malicious actor with various hand-held breaching implements would take to overcome the barrier.[2920] In other words, the facility implements security measures that buy a certain amount of time against malicious actor penetration. No such openly-available security standards are available for labs that do not work with Tier 1 Select Agents. Moreover, openly-available regulations do not stipulate what specific door lock types and door materials are to be employed or avoided for physical barriers to secure a laboratory space.

Access policies make detection of unauthorized individuals easier. Many laboratories provide workers with ID badges and restrict access to the laboratory after normal working hours unless night operations are required for a research project or to provide animal care.[2921] Identification complicates the task of a malicious actor trying to pass as an authorized individual, and restricting operation times decreases the chances that a malicious insider can carry out an act when no one else is around.

Most if not all high containment laboratories have special policies in place to restrict and control visitor access, which in practice often revolve around ensuring that visitors are positively identified in some manner and are escorted on site.[2922]

16.16.3.2 Additional Measure at the Select Agent level

Requirements

BSAT Regulations require that physical security procedures be incorporated into the facility's security plan.[2923] BSAT and animals exposed or infected with a BSAT must be access controlled and secured "against unauthorized access, theft, loss, or release," although the regulations do not detail how this must be done.[2924] "Freezers, refrigerators, cabinets, and other containers where select agents or toxins are stored" must be "secured against unauthorized access," and card access systems and lock boxes are acceptable ways of doing so.[2925]

2920 For use as part of the Select Agent security planning process, see: Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins, Animal and Plant Health Inspection Service (APHIS) Agriculture Select Agent Program, "Security Guidance for Select Agent or Toxin Facilities: 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73," p.30-31, 42-43.
For general use, see for example: Betty E. Biringer, Rudolph V. Matalucci, Sharon L. O'Connor, *Security Risk Assessment and Management: A Professional Practice Guide for Protecting Buildings and Infrastructures* (Hoboken: John Wiley & Sons, Inc., 2007), p. 327.

2921 Ibid, p. 13;
National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version*, p. 258.

2922 LouAnn C. Burnett, "Biosafety Practices Associated with Potential Agents of Biocrime and Biowarfare," Emerging Technologies, Supplement 3, IA.2.4;
Sandia National Laboratories, "Laboratory Biosecurity: A Survey of the U.S. Bioscience Community," p.13.

2923 42 CFR 73.11(c)(1). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

2924 42 CFR 73.11(c)(2) and 42 CFR 73.11(c)(8). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

2925 42 CFR 73.11 (c)(1)-(3). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

The security plan and security training regulations also require measures that ensure a timely and coordinated security response. The facility's security plan must include procedures for "removing unauthorized or suspicious persons," immediately reporting suspicious persons and activities and potential signs of inventory compromise, reporting potential criminal activity to the authorities, and addressing access control compromises (such as lost keys) and access control revocation.[2926]

Implementation at Research Institutions

Several research institutions employee at least three barriers to prevent physical access to the BSAT laboratory. These barriers are controlled using different types of locks to prevent anyone from access if one unlocking mechanism is stolen. These different types can be physical, electronic, human, or physiological.

A certain tradeoff exists between facility security measures and making the facility hard-to-find for external malicious actors, although both measures help ensure physical security of the facility. Employing measures, such as fences, around an institution may enhance physical security, but also draws attention to the facility and singles it out for malicious actors. Some institutions have taken measures to not call attention to buildings wherein BSAT research is conducted to prevent targeting by malicious actors.

Institutions routinely review laboratory access records to identify any anomalies in laboratory access.

The effectiveness of any physical control system is only as good as the responsiveness of the employer to revoke access to ex-employees, particularly in cases where the individual may become malicious as a result of their termination. Modern electronic access control systems often enable a designated security official to disable an individual's access at any time. This security feature is implemented at some campuses, where campus police can shut off access to campus buildings (including laboratories) remotely. One interviewee stated that their institution could immediately shut off building access, at any time, to anyone. Other institutions stated that they could revoke access to the BSAT laboratories within hours if the situation necessitated.

16.16.3.3 Additional Measures at the Tier 1 Select Agent Level

Tier 1 BSAT Regulations require a number of additional physical security measures in addition to those required for Select Agents and Toxins.

A Tier 1 facility must have three security barriers to delay malicious actors. One barrier must be monitored to detect "intentional and unintentional circumventing of established access control measures under all conditions (day/night, severe weather, etc.)" and the final barrier must have some form of access control to ensure that only individuals registered to work with Tier 1 Select Agents are allowed to pass.[2927] Further, procedures must be in place to ensure that powered access control systems maintain continuity of security in the event of a power disruption.[2928] Finally, if the facility is unable to maintain a security force response time at or under 15 minutes, it must have barriers "sufficient to delay unauthorized access until the response force arrives," and therefore be able to prevent "theft, intentional release, or unauthorized access" to all Tier 1 Select Agents.[2929]

Entities with Tier 1 Select Agents are further mandated to restrict access to the laboratory and storage facilities outside of normal business hours by requiring an explicit permission from the facility's

[2926] 42 CFR 73.11 (c)(4)-(8), (d)(7)(i)-(v). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2927] 42 CFR 73.11 (f)(4)(iv). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2928] 42 CFR 73.11(f)(4)(vii). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2929] 42 CFR 73.11(f)(4)(viii)(B). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

responsible official for off-hours access to such facilities.[2930] This access regulation formalizes a good practice introduced in the non-select agent section above.

Physical security at Tier 1 Select Agent facilities is an area of strength in the current regulatory framework. The regulations provide specific metrics the facility must meet, such as the need for a minimum of three physical barriers with certain requirements and a maximum 15 minutes security response time or security barriers adequate to hold off malicious actors until help arrives.[2931] Very detailed guidance has been generated (including a security risk assessment algorithm) to help laboratory managers assess and mitigate security risks.[2932] The regulations are flexible in that laboratories are allowed to determine, with the help of the relevant security providers, what barriers are appropriate to hold off potential malicious actors until help arrives. At the same time, the lab's desired implementation is kept in check through the required licensing process, whereby CDC or APHIS consider the proposed security plan before the facility is allowed to work with a Tier 1 Agent.

Implementation at Research Institutions

Research institutions that are registered for Tier 1 BSAT have at least three barriers in place to ensure regulatory compliance. Some institutions employ more than three barriers. Access to these barriers can be controlled electronically, physically, by humans, or physiologically.

16.16.3.4 Gap Analysis

Based on the above information, the following gaps were identified:

- Current regulatory and guidance documents do not prohibit use of certain insecure physical barriers for non-BSAT laboratories. For example, physical barriers that use simple mechanical keys are insecure and would not necessarily prevent an unauthorized individual from gaining access to a laboratory.[2933] Guidance documents strongly discourage mechanical key locks, and security planning at the Select Agent and Tier 1 Select Agent levels would presumably prevent such setups by arguing that these barriers would not noticeably slow an attacker armed with as little as a crowbar. Discouraging use of inadequate access control measures, such as simple mechanical locks, for all high containment laboratories could help address the gap.

16.16.4 Surveillance and Monitoring

Surveillance and monitoring measures can be used to detect events such as unauthorized entry, exposure to infectious agents, and malfunctioning safety and security equipment. Successful surveillance and monitoring measures enable timely notification of relevant response authorities.

2930 42 CFR 73.11 (f)(4)(ii). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
2931 42 CFR 73.11 (f)(4)(iv), (viii)(B). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
2932 Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins, Animal and Plant Health Inspection Service (APHIS) Agriculture Select Agent Program, "Security Guidance for Select Agent or Toxin Facilities: 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73";
Jennifer Gaudioso, Susan A. Caskey, LouAnn Burnett, Erik Hecgaard, Jeffery Owens, Philippe Stroot, "Strengthening Risk Governance in Bioscience Laboratories."
2933 National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version*, p. 257.

16.16.4.1 General– At All Levels

Requirements

Security-specific surveillance and monitoring measures are not required by regulations for all laboratories.[2934] According to the BMBL, "for laboratories not handling select agents, the access controls and training requirements specified for BSL-2 and BSL-3 in [the] BMBL may provide sufficient security."[2935]

Occupational health monitoring plans are only required for laboratories at the BSL-4 or ABSL-4 levels (see Figure 16.4).[2936] However, biosafety standards make clear that heath surveillance programs are to be put in place if needed based on the type of work conducted at the facility at any level apart from BSL-1 (including ABSL-1). Health surveillance plans, although typically classed as biosafety measures, also have an important biosecurity function in detecting a potential exposure incident. Although the health surveillance program is not designed to discern between deliberate and accidental infections, it would initiate an isolation process, if necessary, and help mitigate the spread of the disease.

Detection of malfunctioning equipment can prevent the occurrence of an incident, or failing this, at least minimize its consequences. Thorough equipment checks are typically conducted once a year during facility shut down. For instance, current OSHA interpretation of regulations require that biosafety cabinets, which play a crucial role in preventing laboratory infection, must be certified when installed, when moved, and at least annually.[2937,2938]

Implementation at Research Institutions

Video surveillance cameras are sometimes present on campuses, at laboratory entrance and exits, in laboratories not working on Select Agents. Although video surveillance is an oft-cited example of a surveillance method, most laboratories do not have the staff nor the budget to monitor video feed in real-time.[2939] Laboratories with video surveillance generally use it for logging purposes only and to assist incident (and accident) investigations. For example, if suspicious activity by an insider is suspected, video logs of their time in the lab can be retrieved and inspected.

Institutions supporting research in high containment laboratories have developed plans for identifying potential exposures and contacting the appropriate health authorities.

Shortly after the Virginia Tech shooting in the mid-2000s, most universities established threat assessment teams to assess potential threats on campus and identify the appropriate approaches and individuals to address the threat. Several institutions have protocols and reporting mechanisms in place to reports

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2934 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 104-105.

2935 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 104-105.

2936 U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 120;
42 CFR 73.12(d). U.S. Government Publishing Office, "Title 42: Public Health, §73.12 Biosafety."

2937 OSHA's interpretation, based on 29 CFR 1030(e)(2)(iii)(B). U.S. Government Publishing Office, "Title 29: Labor, §1910.1030 Bloodborne pathogens," http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=3c86911ee71d159de28dea2738f1d687&r=SECTION&n=se29.6.1910_11030.

2938 OSHA, "OSHA Fact Sheet- Laboratory Safety Biosafety Cabinets (BSCs)," <https://www.osha.gov/Publications/laboratory/OSHAfactsheet-laboratory-safety-biosafety-cabinets.pdf>.

2939 National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version*, p. 258.

incidents that raise concerns. In some cases, these concerns are communicated to the threat assessment teams directly. If a credible or significant threat presents itself, the threat assessments teams can communicate directly with the FBI.

16.16.4.2 Additional Measures at the Select Agent level

Requirements

Surveillance cameras are not explicitly included in the Biological Select Agents and Toxins Regulations. If installed, they must be described in the security plan and do not replace visitor escorts.[2940]

Occupational health monitoring plans are not explicitly included in the Biological Select Agents and Toxin Regulations for any BSAT, including Tier 1 Select Agents. Occupational health monitoring is pathogen-specific, and authoritative guidance exists for specific pathogens.[2941]

Implementation at Research Institutions

Research institutions have installed cameras to monitor access to the BSAT laboratories. This footage is reviewed periodically by authorized institutional administration and security officials.

Several institutions have armed guard patrols to monitor the perimeter of the facility to identify potential security concerns. In addition, if an alarm is triggered at a few of the institutions,

Institutions supporting research in high containment laboratories have employee health monitoring processes and programs regardless of whether the facility works with Select Agents.

Buildings that house animal research and BSAT research conduct perimeter surveillance to identify possible malicious actors. These surveillance efforts are sometimes real-time and involve police patrolling the building or involve periodic review of archived surveillance footage.

16.16.4.3 Additional Measures at the Tier 1 Select Agent Level

Requirements

An intrusion detection system must be placed in all places that house or work with Tier 1 Select Agents or that "reasonably afford access" to such spaces, unless these zones are physically occupied.[2942]

Tier 1 BSAT Regulations specify that all individuals with access to Tier 1 Select Agents must be enrolled in an occupational health program.[2943]

Implementation at Research Institutions

Because none of the laboratories we visited were Tier 1 Select Agent laboratories, no specific information was collected on surveillance and monitoring of Tier 1 facilities. However, surveillance efforts would be more stringent than what is currently implemented for BSAT laboratories.

2940. Federal Select Agent Program, "Security Guidance for Select Agent or Toxin Facilities," <http://www.selectagents.gov/resources/Security_Guidance_v3-English.pdf>.
2941. 42 CFR 73.12(d). U.S. Government Publishing Office, "Title 42: Public Health. §73.12 Biosafety"; Centers for Disease Control, "Appendix F6 – Guidelines for Medical Surveillance of Laboratory Personnel Working with SARS-CoV," <http://www.cdc.gov/SARS/guidance/f-lab/app6.html>.
2942. 42 CFR 73.11(f)(4)(v). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
2943. 42 CFR 73.12(d). U.S. Government Publishing Office, "Title 42: Public Health, §73.12 Biosafety."

16.16.4.4 Gap Analysis

Based on the above information, the following gaps were identified:

- The length of time between reviews of footage from video surveillance could prevent rapid identification, prevention, or response to an incident involving unauthorized access or an actual event. However, the effectiveness of real-time video may not be significant.[2944]

16.16.5 Storage, Inventory, and Accountability Processes

Inventory and material management processes allow labs to keep track of biological materials. Keeping track of these materials enables loss or theft detection, which can assist in post-event investigations.

16.16.5.1 General– At All levels

Requirements

The BMBL recommends some form of "inventory or material management process for control and tracking of biological stocks or other sensitive materials" as part of a general biosafety program.[2945]

Implementation at Research Institutions

Some research institutions routinely check that all pathogen stocks are accounted for. Several high containment laboratories keep records of stored pathogens. Some institutions lock freezers used to store their pathogen stocks.

Several laboratories do not do not allow live or active pathogens from being removed from high containment without adequate fixation, inactivation, or decontamination.

16.16.5.2 Additional Measures at the Select Agent level

Requirements

Inventory control measures and procedures for reporting and responding to the detected alteration of inventory records must be codified in the security plan required as part of the Select Agents registration process.[2946] Select Agent regulations stipulate that detailed inventory records be kept for each Select Agent held in long-term storage, including the name, number of containers, storage location, and chain-of-custody information.[2947] This does not apply to working stocks (i.e., less than 30 days, like inoculated cells or aliquots diluted to working concentration and intended for use in the near future.[2948] Working

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[2944] Even the DOD 2009 report, which stressed the value of "enhanced and increased video monitoring of the labs," did not call for continuous real-time video surveillance. p.20-23, Department of Defense, Defense Science Board Task Force, "Department of Defense Biological Safety and Security Program," May 2009, Unclassified, Cleared for Public Release, http://www.acq.osd.mil/dsb/reports/ADA499977.pdf; National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version*, p. 258.

[2945] U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories – Fifth Edition*, p. 105.

[2946] 42 CFR 73.11(c)(1), (c)(6), (d)(7)(v). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

[2947] Volumes must be recorded for toxins only, not agents.
42 CFR 73.17(a)(1), (a)(5). U.S. Government Publishing Office, "Title 42: Public Health, §73.17 Records," <http://www.ecfr.gov/cgi-bin/retrieveECFR?r=PART&n=42y1.0.1.6.61>.

[2948] CDC, APHIS, "Guidance on the Inventory of Select Agents and Toxins," p. 6, <http://www.selectagents.gov/resources/Long_Term_Storage_version_5.pdf>

stocks must be kept in an access controlled area registered with the Federal Select Agent Program.[2949] An inventory of infected or exposed animals also must be recorded for use in case of escape or theft; the recorded information includes the quantity, species, location, and final disposition of the animal.[2950]

The Select Agent regulations require routine inventory audits of pathogens in long-term storage. Inventory audits must be performed when a collection of Select Agents is moved, when a principal investigator working with Select Agents leaves or joins the lab, and in the event of theft or loss (at which point all stocks of agents under the principal investigator responsible for the missing stock are to be audited).[2951]

In addition to pathogen accounting, entry and exit to areas holding Select Agents must be recorded, including the name of the individual, the name of their escort if applicable, and the date and time of entry.[2952] Furthermore, all records stipulated by the Select Agents regulations must themselves have "controlled access" and must be in such a form that "their authenticity may be verified."[2953]

Under current Select Agent regulation 42 CFR 73.11(d)(3), a lock box system is explicitly suggested, alongside card systems, as a means of meeting the requirement for "freezers, refrigerators, cabinets, and other containers where select agents or toxins are stored to be secured against unauthorized access."[2954] The BSAT Regulations explicitly allow lock and key systems as a means of ensuring access control to long-term pathogen storage.[2955] Unlike lock boxes, many electronic systems (numeric, card, and biometric) automatically log the user and the time of access. Electronic logging of such information would help detect anomalous behavior, such as the opening of a container by an individual at abnormal hours, and assist in investigating incidents by having an (additional) electronic record of everyone who accessed Select Agents. These systems enhance an institute's capability to keep required records regarding select agent stocks in long-term storage and "information about all entries into areas containing select agents or toxins."[2956]

Implementation at Research Institutions

Institutions that support BSAT research adhere to the federal guidance on long-term storage of BSAT. Some of the institutions use an automated inventory system where all vials have a bar code. Others secure pathogens in boxes with security tape to know which boxes have been touched. The freezers are locked. In addition, several institutions keep paper inventory logs.

Institutions varied in their inventory checks. Some conducted checks on a routine cycle, while others conducted random inventory checks in addition to the periodic checks. Institutions assess inventory if a vial appears to be missing.

[2949] CDC, APHIS, "Guidance on the Inventory of Select Agents and Toxins," p. 6, <http://www.selectagents.gov/resources/Long_Term_Storage_version_5.pdf>.
[2950] CDC, APHIS, "Guidance on the Inventory of Select Agents and Toxins," p. 6, <http://www.selectagents.gov/resources/Long_Term_Storage_version_5.pdf>.
[2951] 42 CFR 73.11(e)(1)-(3). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2952] 42 CFR 73.17(a)(5). U.S. Government Publishing Office, "Title 42: Public Health, §73.17 Records."
[2953] 42 CFR 73.17(a)(7)(b). U.S. Government Publishing Office, "Title 42: Public Health, §73.17 Records."
[2954] 42 CFR 73.11(d)(3). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security," <http://www.ecfr.gov/cgi-bin/text-idx?SID=94bd3a730b8387eb15bc058bc4637627&mc=true&node=se42.1.73_111&rgn=div8>.
[2955] 42 CFR 73.11(d)(3). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2956] 42 CFR 73.17(a)(1), (a)(5). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Records," <http://www.ecfr.gov/cgi-bin/retrieveECFR?r=PART&n=42y1.0.1.6.61>.

16.16.5.3 Additional Measures at the Tier 1 Select Agent level

Requirements

No additional storage, inventory, and accountability processes are required for Tier 1 Select Agents that go beyond those stipulated BSAT.[2957] However, Tier 1 Select Agents regulations contain a general clause that: "an entity's Responsible Official will coordinate their efforts with the entity's safety and security professionals to ensure security of Tier 1 select agents and toxins and share, as appropriate, relevant information."[2958]

Implementation at Research Institutions

Because none of the laboratories we visited were Tier 1 Select Agent laboratories, no specific information was collected on surveillance and monitoring of Tier 1 facilities.

16.16.5.4 Gap Analysis

Based on the above information, the following gaps were identified:

- Inventory measures and audits facilitate detection of discrepancies and use patterns that may indicate theft. However, no practical ways exist to measure and track working stocks. No practical methods exist that would provide accurate working stock pathogen inventory data. Required accountability checks verify container counts, but recording volume or pathogen concentrations is not required. Volumes and pathogen concentrations are often recorded by researchers for experimental purposes, but this is not part of the traceable inventory process. Practitioners have repeatedly argued that, "beyond knowing who has what pathogen, exact inventory rules are not informative or feasible, particularly for pathogens actively being experimented [upon]."[2959,2960] The inability to maintain an accurate inventory of working stocks cannot be resolved. Therefore, working stock control must rely on physical security and personnel reliability.

16.16.6 Transfer, Shipment, and Chain-of-Custody Protocols

Secure transfer of pathogens involves: 1) ensuring proper documentation and approvals are provided; 2) not alerting anyone to the contents of the package during shipment, if relevant; and 3) comprised of means to detect, report, and respond to missing or damaged packages. Other than clinical and diagnostic samples, pathogens are rarely shipped. In addition, recent incidents of accidental shipment of live or incorrect pathogens have resulted in only a few transportation companies willing to ship infectious agents.

[2957] U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2958] 42 CFR 73.11 (f)(2). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."
[2959] AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Implementing the Revised Select Agents and Toxin Regulations Proceedings from the Meeting*, p. 15.
[2960] Nancy Connell, "Biological Agents in the Laboratory- The Regulatory Issues

16.16.6.1 General– At All levels

Requirements

Department of Transportation regulations categorizes infectious substances as "Division 6.2" goods for shipment under transport regulations, and further divided into two categories (A and B).[2961] Category A is for an "infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs," whereas Category B is for any other infectious substance.[2962] Classification of an infectious substance as Category A or B "must be based on the known medical history or symptoms of the source patient or animal, endemic local conditions, or professional judgment concerning the individual circumstances of the source human or animal."[2963]

Both Category A and B substances are triple packaged for transport, although Category A packaging requirements are more stringent in terms of leak resistance.[2964,2965] Air shipment requires that at least one side of the external layer be emblazoned with an "Infectious Substances" diamond hazard label.[2966,2967,2968,2969] Although transportation regulations require a security transport plan for a hazardous goods shipment, "Division 6.2" goods are not covered by these regulations.[2970]

The CDC and USDA require permits to import pathogens.[2971,2972,2973] USDA also requires a permit to ship

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2961 U.S. Government Publishing Office, "Title 49 Transportation, §173.134 Class 6, Division 6.2 – Definitions and exceptions," <http://www.ecfr.gov/cgi-bin/text-idx?SID=c43d9605516b239af6c12f288eef1a86&mc=true&node=se49.2.173_1134&rgn=div8>. Similar categorization of agents is included in the International Air Transport Association's Dangerous Good Regulations.

2962 49 CFR 173.134 (a)(1)(i)-(ii). U.S. Government Publishing Office, "Title 49 Transportation, §173.134 Class 6, Division 6.2 – Definitions and exceptions."

2963 U.S. Government Publishing Office, "Title 49 Transportation, §173.134 Class 6, Division 6.2 – Definitions and exceptions."

2964 For instance, if shipped at ambient temperatures or higher, Category A substances must have a positive means of ensuring a leakproof seal.
U.S. Government Publishing Office, "Title 49 Transportation, 173.196 Category A infectious substances," http://www.ecfr.gov/cgi-bin/text-idx?SID=c43d9605516b239af6c12f288eef1a86&mc=true&node=se49.2.173_1134&rgn=div8.

2965 U.S. Government Publishing Office, "Title 49 Transportation, 173.199 Category B infectious substances," <http://www.ecfr.gov/cgi-bin/text-idx?SID=8dd11b1ac9a22e45afc96ac5b2489afe&mc=true&node=pt49.2.173&rgn=div5#se49.2.173_1199>

2966 U.S. Department of Transportation, Pipeline and Hazardous Materials Safety Administration (PHMSA), "Transporting Infectious Substances Safely," October 1, 2006, listed as of August 2015 as current on PHMSA website, <http://www.phmsa.dot.gov/pv_obj_cache/pv_obj_id_54AC1BCBF0DFBE298024C4C700569893C2582700/filename/Transporting_Infectious_Substances_brochure.pdf>,

2967 http://phmsa.dot.gov/portal/site/PHMSA/menuitem.6f23687cf7b00b0f22e4c6962d9c8789/?-vgnextoid=4d1800e36b978410VgnVCM100000d2c97898RCRD&vgnextchannel=0f0b143389d8c010VgnVCM1000008049a8c0RCRD&vgnextfmt=print.

2968 UPS, "Infectious Substances, Category A," <http://www.ups.com/content/us/en/resources/ship/hazardous/responsible/diagnostic.html>;

2969 University of Virginia, "Shipping Infectious Substances by Air," <http://ehs.virginia.edu/biosafety/bio.transport.air.html>

2970 U.S. Government Publishing Office, "Title 49 Transportation, 172 Subpart I- Safety and Security Plans," <http://www.ecfr.gov/cgi-bin/text-idx?SID=1530a4d53604eb26660f7b121832fd2d2&mc=true&node=sp49.2.172.i&rgn=div6>

2971 CDC issues permits for human pathogens and USDA issues permits for animal and plant pathogens.

2972 9 CFR 122, 42 CFR 71.54. U.S. Government Publishing Office, "Title 9 Animals and Animal Products, Part 122 Organisms and Vectors," <http://www.ecfr.gov/cgi-bin/text-idx?SID=fb98f5f1a14891a6cbd139179bc3dfae&mc=true&node=pt9.1.122&rgn=div5>;

2973 U.S. Government Publishing Office, "Title 42 Public Health, Part 71 Foreign Quarantine, Subpart 71.54 Import regulations for infectious biological agents, infectious substances, and vectors." <http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=e170ce9bdb27d491a0e1d31d7bffeb21&ty=HTML&h=L&r=SECTION&n=42y1.0.1.6.59.6.19.5%20%28>

pathogens across state lines, and the CDC sometimes requires a permit to transfer imported pathogens across state lines.[2974] The CDC also provides detailed instructions on safe packaging of infectious substances for shipment.

Similarly, international export of biological agents may require a Department of Commerce export control permit if they are restricted and not exempt. In addition, a Department of State registration and permit may be needed if the agent falls within the ITAR regulations for arms control.[2975,2976,2977,2978] Commerce regulations apply to pathogens restricted by the Australia Group and Select Agents and State regulations apply to biological agents "modified to increase…capability to produce casualties in humans or livestock."[2979]

Implementation at Research Institutions

Institutions appear to require hazardous materials shipping training and certification for all employees who are designated as shippers.[2980] In addition, institutions have offices dedicated to ensuring compliance with all export control regulations. Some institutions have a designated individual, "Export Controls Coordinator," to provide assistance with the export control requirements.[2981]

16.16.6.2 Additional Measures at the Select Agent level

Requirements

The transfer of BSAT between separate entities licensed to possess BSAT requires prior approval by either the CDC or APHIS unless the Select Agent is contained in a specimen for proficiency testing. In the latter case, the CDC or APHIS must simply be informed at least seven calendar days prior to the transfer.[2982]

For all transfers of BSAT, the recipient must keep records of shipments and report to CDC or APHIS within 48 hours after the slated delivery time that the shipment has been received as planned, or that it is delayed or missing.[2983] Furthermore, the recipient must immediately report to the CDC or APHIS if the

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2974 U.S. Government Publishing Office, "Title 42 Public Health, Part 71 Foreign Quarantine, Subpart 71.54 Import regulations for infectious biological agents, infectious substances, and vectors." Center for Disease Control and Prevention. Interstate Shipment of Etiologic Agents. Accessed on http://www.cdc.gov/vaccines/pubs/surv-manual/appx/appendix24-etiologic-agent.pdf. Accessed on September 19, 2015.

2975 Usually "fundamental research" is exempted from the Commerce permits, but not in the cases of biological weapons potential or restricted publication of results.

2976 15 CFR 734.3-8. U.S. Government Publishing Office, "Scope of the Export Administration Regulations." <http://www.gpo.gov/fdsys/pkg/CFR-2001-title15-vol2/pdf/CFR-2001-title15-vol2-part734.pdf>.

2977 15 CFR 744.4-6. U.S. Government Publishing Office, "Control Policy: End-User and End-Use Base," <http://www.gpo.gov/fdsys/pkg/CFR-2001-title15-vol2/pdf/CFR-2001-title15-vol2-part744.pdf>

2978 22 CFR 121.1(XIV)(b) U.S. Government Publishing Office, "The United States Munitions List," <http://www.ecfr.gov/cgi-bin/text-idx?SID=88e7fab9254a3319c3df6fb11a2233ab&mc=true&node=se22.1.121_11&rgn=div8>

2979 U.S. Department of Commerce, *Commerce Control List*, "Category 1 – Special Materials and Related Equipment, Chemicals, 'Microorganisms' and 'Toxins'," <*http://www.bis.doc.gov/index.php/forms-documents/doc_download/989-ccl1*>.

2980 For example: University of California, Irvine, Environmental Health and Safety, "Shipper's Responsibilities," <http://www.ehs.uci.edu/programs/dgoods/>.

2981 For example: University of Colorado Boulder, Office of the Vice Chancellor for Research, Research Administration and Support, "ORI (Compliance), Export Controls, Guidance, Biological Agents," <http://www.colorado.edu/vcr/export-controls/guidance/biological-agents>.

2982 The transfer request is made using APHIS/CDC Form 2. U.S. Government Publishing Office, "Title 42: Public Health, §73.16 Transfers."

2983 42 CFR 73.16(j). U.S. Government Publishing Office, "Title 42: Public Health, §73.16 Transfers."

package was damaged to the point that a release may have occurred.[2984]

The same Category A and B packaging rules described above apply for all pathogens. That is, a package carrying a Select Agent is visually indistinguishable from a package carrying a pathogen not on the Select Agent list.

Finally, Select Agent regulations have a clause regarding suspicious packages. Any suspicious packages must be inspected "before they are brought into or removed from the area where select agents or toxins are used or stored."[2985] The rationale behind this regulation is that a suspicious package may contain an explosive device, which could then potentially breach containment.[2986]

Implementation at Research Institutions

All packages are prepared by a BSAT approved individual, transferred to the shipper in person, and received from the shipper in person by a BSAT approved individual. Chain-of-custody is maintained throughout. Once a package is received by the recipient institution, its outer packaging is examined for any damage before it is transported to the recipient laboratory's containment facility wherein the package's contents will be examined for any damage. The CDC and shipper are notified immediately if the package is damaged.

Transportation security measures must balance the desire for additional security measures against the desire to avoid drawing attention to a particular shipment. Select Agent shipments that are visually indistinguishable from any other shipments of infectious substances once packaged limits the risk of highlighting the package with the restricted BSAT. Knowing the dates of shipment, the identification of the exact trucks carrying the virus, and transportation lines used is impossible without access to the shipping and tracking information.

Very few pathogen shipments occur each month, which is confirmed by the CDC and APHIS joint informational website, which provides additional information on transfer frequency and security. The webpage on BSAT states that "approximately 4250 transfers that have occurred since 2003," with "one confirmed loss of a select agent that occurred during shipment."[2987] The FBI investigation that resulted "determined that the loss most likely did not occur at either the shipping or receiving areas," (i.e., that the package was apparently lost during the transit portion itself).[2988] Furthermore, GoF viruses apparently are not shipped.

Interviewees further noted that the Department of Transportation did surprise inspections to ensure that transfers of pathogens were conducted according to the regulations.

2984 Ibid.

2985 42 CFR 73.11(d)(4). U.S. Government Publishing Office, "Title 42: Public Health. §73.11 Security."

2986 Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins, Animal and Plant Health Inspection Service (APHIS) Agriculture Select Agent Program. "Security Guidance for Select Agent or Toxin Facilities: 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73," July 5, 2013, p.23-24, 38, <http://www.selectagents.gov/resources/Security_Guidance_v3-English.pdf>.

2987 CDC, APHIS. "Federal Select Agent Program Guidance on the Shipment and Receipt of Packages with Select Agents and Toxins," http://www.selectagents.gov/guidance-shipreceipt.html.

2988 CDC, APHIS. "Federal Select Agent Program Guidance on the Shipment and Receipt of Packages with Select Agents and Toxins."

16.16.6.3 Additional Measures at the Tier 1 Select Agent level

Requirements

No additional regulations exist for transport of Tier 1 Select Agents beyond those applicable for all BSAT.[2989] The same Category A and B packaging rules described above apply for all pathogens. As a result, a package carrying a Tier 1 Select Agent should be visually indistinguishable from one carrying a Select Agent pathogen not also a Tier 1 Select Agent, or one carrying a pathogen that is not a Select Agent.

Implementation at Research Institutions

Because none of the laboratories we visited were Tier 1 Select Agent laboratories, no specific information was collected on surveillance and monitoring of Tier 1 facilities.

16.16.6.4 Gap Analysis

Based on the above information, the following gaps were identified:

- Best practices for transportation security are not public, if they exist. General methods to mitigate transportation vulnerability include ensuring that the transport has GPS tracking and a transport-based alert system that contacts police in case of an emergency (readily available in retail and armored transport vehicles).[2990,2991] Another vulnerability-reducing approach is to ensure that the transporting company monitors have the appropriate points of contact to quickly relay information to the appropriate law enforcement agency. Providing an approach through which practitioners can share best practices could enhance transportation security.

16.16.7 Emergency Response Protocols

Emergency response plans, drills, and notification systems prepare research facilities to respond to all-hazards emergencies, including security emergencies.

16.16.7.1 General– At All levels

Requirements

All laboratories should have a general emergency plan as part of their OSHA worker safety requirements, at the very least to deal with fires and with natural emergencies (such as earthquakes, tornadoes, or floods).

[2989] CDC, APHIS, "Federal Select Agent Program Guidance on the Shipment and Receipt of Packages with Select Agents and Toxins."

[2990] For examples in common use, see, Rory Reid, "Citroen eTouch emergency panic button calls cops automatically," *CNET*, October 5, 2010, <http://www.cnet.com/news/citroen-etouch-emergency-panic-button-calls-cops-automatically/>; "Avis 'Panic Button' Debuts in Miami Cars," *Orlando Sentinel*, September 14, 1994, <http://articles.orlandosentinel.com/1994-09-14/business/9409130599_1_gundestar-avis-emergency-button>.

[2991] For features commercially available in the high-security transport field, see for example: 3SI Security Systems, "Cash-in-Transit (CIT) Tracker™," <https://www.3sisecurity.com/products/en-cash-in-transit-cit-tracker/>

Implementation at Research Institutions

Due to concerns about active shooters on the institution's property and the consequences of natural disaster, research institutions plan and conduct large and small-scale exercises to practice response and identify potential areas for improvement. Small-scale exercises include relevant members of the institution whereas large-scale exercises involve local police and first responders and FBI. Institutions practice a wide range of exercises to make sure that the appropriate institutional officials know what to do and with whom to communicate in an emergency situation.

In addition, all building, electrical, and safety equipment are tested periodically.

Some institutions had emergency operations centers to facilitate communication and coordinate response efforts in an emergency.

16.16.7.2 Additional Measures at the select agent level

Requirements

Annual drills are required to test emergency and incident response plans.[2892]

Implementation at Research Institutions

An effective emergency response depends on appropriate planning to ensure that the response is coordinated and appropriate for the situation, lines of communication with the laboratory to ensure the safety of laboratory personnel, appropriate equipment, and familiarity with using said equipment and the laboratory layout. Given the complexity of responding to security situations at a high-containment laboratory, law enforcement is actively involved with laboratory-organized security training exercises.

All BSAT institutions involve laboratory staff to practice responses to small-scale incidents that occur in the laboratory, such as spills. These small-scale exercises are sometimes conducted a few times a year. In addition, several institutions conduct medium-sized exercises featuring rotating scenarios with institutional or local law enforcement and first responders to ensure all individuals are property trained to respond to different types of emergencies.

16.16.7.3 Additional Measures at the Tier 1 Select Agent level

Requirements

Entities with Tier 1 Select Agents must have a security response time at or below 15 minutes, or otherwise provide barriers that are "sufficient to delay unauthorized access until the response force arrives."[2893] A facility's security response time is measured starting from the tripping of an intrusion alarm or incident report, to the arrival of the security force to the first security barrier.

Implementation at Research Institutions

The institutions that support Tier 1 BSAT conduct exercises with institutional and/or local law enforcement and first responders to test response activities and ensure that all individuals have the proper information and training to safely respond to emergency situations.

[2892] 42 CFR 73.14(f). U.S. Government Publishing Office, "Title 42: Public Health, §73.14 Incident response."
[2893] 42 CFR 73.11(f)(4)(viii)(A). U.S. Government Publishing Office, "Title 42: Public Health, §73.11 Security."

16.16.7.4 Gap Analysis

We did not identify any gaps in emergency response. Based on our discussions, institutional, local, and federal law enforcement and relevant institutional officials appear to identify productive ways of working together in different scenarios.

However, we identified a gap when discussing exercises and drills to practice emergency response. The degree to which facilities conduct exercises on security-related incidents varies. Exercise topics include response to fires and bombs, natural disasters, and biosafety incidents, such as spills. The nature of the exercise planning process and the participation of local first responder agencies varies by exercise and facility.

16.16.8 Indirect Security Measure: Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA

Although the Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA does not require security practices to be implemented at research institutions, it is included in this governance section as an indirect measure. Concerns about development of biological agents using chemical synthesis of viral genomes have driven the development of this Guidance. GoF viruses are generated in the laboratory using genetic engineering and, in some laboratories, synthetic genomics.

Concerns about chemical synthesis of pathogen genomes and the ability to purchase virulence genes and genes from Biological Select Agents and Toxins raised significant concern among the security policy community in the early 2000s. During this time, the US government and international community were evaluating the potential for life science and biotechnology to enable both beneficial and destructive research and two research groups published scientific articles on chemical synthesis of infectious human and bacterial viruses, both of which were carried out using DNA molecules purchased from DNA synthesis providers.[2994] The publication of these papers led to concerns within the security and security policy communities about creation of viral genomes, particularly of viruses on the Biological Select Agents and Toxins list. These concerns prompted the NSABB to evaluate the biosecurity considerations associated with synthesis of Biological Select Agent Toxins and recommend approaches to address any risks.[2995] Three of NSABB's recommendations were:

1. The Departments of Health and Human Services and Agriculture develop "harmonized guidance to investigators and nucleic acid/gene/genome providers concerning the SAR [Select Agent Regulations] with respect to synthetically-derived DNA."

2. The federal government develop a process that providers can use to screen for Biological Select Agents and Toxins, develop and promote "preferred practices for screening orders and interpreting results," among other related activities, and

3. Evaluate current biosafety guidelines to ensure that guidelines and regulations are adequate for synthetically derived DNA.

[2994] At that time, a fairly new industry of gene synthesis providers had developed to provide the service of making genes from DNA sequences submitted by its customers. The field was enabled by technologies that allow for long pieces of DNA to be made chemically and with high fidelity.

[2995] National Science Advisory Board for Biosecurity. Addressing Biosecurity Concerns Related to the Synthesis of Select Agents. Dec 2006. Accessible at http://osp.od.nih.gov/sites/default/files/resources/Final_NSABB_Report_on_Synthetic_Genomics.pdf. Accessed on September 18, 2015.

In 2010, the Department of Health and Human Services issued its Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA.[2996] This is a voluntary guidance that includes provisions for screening of sequences and customers. The overarching goal of the Guidance is to "minimize the risk that unauthorized individuals or individuals with malicious intent will obtain "toxins and agents of concern" through the use of nucleic acid synthesis technologies and to simultaneously minimize any negative impacts on the conduct of research and business operations." The Guidance states that companies should screen customers to verify their identity and legitimacy, identify any "red flags," and ensure all US trade and export control regulations are followed. Sequence screening involves evaluating the order sequence to determine whether it is more similar to a sequence from a Biological Select Agent or Toxin than it is to a sequence from an organism not on that list. If it is, the Guidance states that the company should conduct follow-up screening to "verify" that the customer has a legitimate use of the gene and "is acting within their authority." In addition, the Guidance provides resources to gene synthesis providers to assist in consulting the appropriate US regulations or guidance, contact the Federal Bureau of Investigation Weapons of Mass Destruction Unit if any concerns arise, and consult with the Select Agent Program and Department of Commerce if questions arise.

In practice, the gene synthesis industry has changed since the Guidance has been released. A series of commercial acquisitions have changed the landscape of gene synthesis companies where many of the companies engaged in the development of the NSABB recommendations and US government guidance have been consolidated. Other companies, not previously engaged seem to have appeared in this space. In addition, companies from China seem to have become engaged in the international consortiums for gene synthesis companies. At least two international industry associations have emerged and both have discussed and encouraged their members to screen sequences and customers. Members of the International Gene Synthesis Consortium (IGSC) have formed a non-profit corporation to make it easier for small companies, non-profit organizations, and academic institutions to "leverage the biosecurity expertise of the IGSC."[2997]

16.16.9 Governance of Hazardous Chemicals

Life science research often involves use of hazardous chemicals. Many of these chemicals are toxic and some are flammable, reactive, or explosive.[2998] These chemicals could be misused by a malicious actor to facilitate other malicious acts, including arson, bombing, and sabotage.

Regulations and best practices governing the storage and use of hazardous chemicals limits the ability of malicious actors to divert hazardous supplies already present within the laboratory to carry out malicious acts. Hazard communication regulations require the labelling of hazardous chemicals, and stipulate that employees must be made aware of chemical hazards through training.[2999]

Regulations explicitly require that laboratories must "minimize all chemical exposures and risks."[3000] Chemical risk mitigation is done at the facility level through a Chemical Hygiene Plan, which specifies

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[2996] Department of Health and Human Services. Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA. 2010. Accessible at http://www.phe.gov/Preparedness/legal/guidance/syndna/Documents/syndna-guidance.pdf. Accessed on September 18, 2015.

[2997] Schubert E. International Gene Synthesis Consortium Forms Not-for-Profit Corporation. April 28, 2015. PRLOG. Accessible at http://www.prlog.org/12450359-international-gene-synthesis-consortium-forms-not-for-profit-corporation.html. Accessed on September 18, 2015.

[2998] National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version*. p. 53-74.

[2999] U.S. Government Publishing Office, "Title 29 Labor, §1910.1200 Hazard Communication," <http://www.ecfr.gov/cgi-bin/text-idx?rgn=div8&node=29:6.1.1.1.1.1.1.36>.

[3000] U.S. Government Publishing Office, "Title 29 Labor, §1910.1450 Occupational exposure to hazardous chemicals in laboratories," <http://www.ecfr.gov/cgi-bin/text-idx?rgn=div8&node=29:6.1.1.1.1.1.1.36>.

what measures the employer will take to protect the employees from chemical hazards.[3001] Regulations further require that any work with hazardous chemicals must be preceded by a risk assessment, which will "identify chemicals to be used, amounts required, and circumstances of use in the experiment."[3002] The National Research Council of the National Academies (NRC), funded by NIH, has provided extensive guidance on minimizing chemical hazards.[3003]

In addition, institutions possessing sufficient quantities and types of chemicals that are covered by the Chemical Facility Anti-Terrorism Standards must also comply with its personnel, physical, and inventory security requirements. However, many universities are exempt from these requirements because they do not possess the minimum quantity of chemicals as stipulated in the Standards.

16.16.10 Gaps in Security Governance

In addition to gaps described in the previous section, the following overarching issues were identified:

16.16.10.1 Financial and Technical Resources

The level of financial and technical resources made available to maintain Select Agent facilities at a high security level in light of stricter regulations is of significant concern. Regulations have become stricter to meet growing security concerns. At the same time, few additional financial, administrative, and informational resources have been made available for laboratories to meet these new requirements.[3004] Institutional administrators have repeatedly raised these issues in light of decreased state funding and attrition of select agent facility staff.[3005] Without sufficient program funds, institution managers will have to implement cuts elsewhere to meet the minimum regulatory requirements. For instance, the number of full-time biosafety employees may be reduced.[3006] Furthermore, this situation is significantly exacerbated by the current level of regulatory burden facing research institutions. The only known estimate of cost burden was produced by the Federal Select Agent Program before the most recent regulatory changes to the BSAT Regulations.[3007] However, to the best of our knowledge, no other assessments that quantify time spent, financial cost of implementing the regulations, and opportunity costs exist.

16.16.10.2 Lack of Clarity About Requirements

A lack of clarity about effective practices to implement the security requirements exists. In some cases, such as for personnel security, the Federal Select Agent Program has issued a guidance document. However, the continuously changing regulatory environment, variability across inspections, and the lack

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3001 U.S. Government Publishing Office, "Title 29 Labor, §1910.1450 Occupational exposure to hazardous chemicals in laboratories."

3002 U.S. Government Publishing Office, "Title 29 Labor, §1910.1450 Occupational exposure to hazardous chemicals in laboratories."

3003 National Research Council of the National Academies, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards, Updated version*.

3004 AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Implementing the Revised Select Agents and Toxin Regulations Proceedings from the Meeting*, p. 13-15.

3005 Ibid.

3006 A practitioner survey conducted in 2008 found that at the BSL-3/ABS-3 laboratory level, more than half (64%) of the respondents indicated their facility operated with less than three full-time equivalent employees devoted to biosafety. Allison T. Chamberlain et al., "Biosafety Training and Incident-reporting Practices in the United States: A 2008 Survey of Biosafety Professionals," Applied Biosafety 14, no. 3 (2009): p. 138, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2947438/>.

3007 Centers for Disease Control and Prevention and U.S. Department of Agriculture. Regulatory Impact Analysis & Final Regulatory Flexibility Analysis. 2011. Accessible at http://system.suny.edu/media/suny/content-assets/documents/compliance/ehs/Regulatory-Impact-Analysis-and-Final-Reg-Flexibility-Analysis.pdf. Accessed on September 19, 2015.

of a forum through which to discuss best practices for biological laboratory security, presents a gap between the requirement, regulators, and regulated community. Efforts to identify and resolve specific problems to enhance security beyond compliance checklists could help address gaps specific to certain security measures.

Confusion on regulations is detectable from the lack of consistency between inspection results from the same facility, where different inspectors interpret existing regulations differently.[3008] In one case, a laboratory was cited for not having provided workers with animal subjects training when the laboratory did not conduct work with animals.[3009] Such uncertainty could lead to laboratory managers dedicating resources to meeting interpretations of the legislation in an effort to avoid regulatory trouble, resulting in a negative impact on security-relevant spending. Unclear regulations could also risk seeing objectively unsatisfactory, but technically correct implementation.

Results from inspections carried out by the Office of the Inspector General of the Department of Health and Human Services in the 2003– 2005 period demonstrated that a significant time gap could exist between the roll-out of new regulations and satisfactory implementation across all concerned institutes. These inspections demonstrated gaps in implementation of regulations at some institutes, ranging from weaknesses in access controls, to insufficient security plans and incident response plans.[3010,3011,3012,3013] The current environment of diminished resources and a lack of consensus in regulatory interpretations would probably slow implementation of any further regulations and potentially impedes current implementation of regulations.

16.16.10.3 Guidance on Integrating Cross-Over Biosafety and Biosecurity Measures

Appropriate guidance was not identified for integrating biosafety and biosecurity measures, such as waste management systems. Harmonization of guidance for these measures would enhance biological safety measures to prevent its exploitation and resolve discrepancies between practice and biosafety/biosecurity objectives. Examples exist where practices sufficient for promoting biosafety may present opportunity for a malicious actor.

16.16.10.4 Regulatory Nomenclature of Pathogens and Toxins

Nomenclature issues with respect to infectious diseases may lead to confusion. The use by different branches of the federal government of several tier lists and risk categories for pathogen characterization is a potential area of confusion, with potential negative repercussions on laboratory compliance. For example, USDA/APHIS has a list entitled "High-Consequence Foreign Animal Diseases and Pests" in addition to the more established animal and plant "Select Agents" list. Within both of these lists exists a subset of pathogens ("Tier 1") described to present significant security threats. On top of these

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3008 Practitioners have argued that inspections "rely heavily on individual inspector interpretations of the regulations." AAAS, AAU, APLU, FBI, *Bridging Science and Security for Biological Research: Implementing the Revised Select Agents and Toxin Regulations Proceedings from the Meeting*, p. 14-15.

3009 Ibid.

3010 Daniel R. Levinson, Inspector General, Office of the Inspector General, Department of Health and Human Services, "Summary Report on State, Local, Private, and Commercial Laboratories' Compliance With Select Agent Regulations (A-04-06-01033)," January 9, 2008, p. i-ii, <http://oig.hhs.gov/oas/reports/region4/40601033.pdf>;

3011 Daniel R. Levinson, "Summary Report on Universities' Compliance with Select Agent Regulations (A-04-05-02006)," June 30, 2006, <http://oig.hhs.gov/oas/reports/region4/40502006.pdf>

3012 Dara Corrigan, Acting Principal Deputy Inspector General, "Summary Report on Select Agent Security at Universities (A-04-04-02000)," May 25, 2004, <http://oig.hhs.gov/oas/reports/region4/40402000.pdf>;

3013 See also the list of cases in the Sandia National Laboratory report SAND2009-8070: Jennifer Gaudioso, Susan A. Caskey, LouAnn Burnett, Erik Heegaard, Jeffery Owens, Philippe Stroot, "Strengthening Risk Governance in Bioscience Laboratories," p.81-94.

designations, separate "Category A and B" designations are used by both the Department of Transportation and NIH/NIAID are of different composition and used for different purposes despite similar terminology. Keeping the agents, lists, and designations straight may present challenges to individuals and institutions who are complying with requirements from several or all of these departments and agencies.

16.16.11 Major Challenges

The major challenges emerging from the evaluation are:

- Research involving infectious disease and animal research are governed by numerous Executive Orders, laws, guidance, and contractual requirements at the federal level. In general, this tapestry of governance appears to be effective at preventing/mitigating physical security risks. However, of all required security measures, personnel security (i.e., identifying, assessing, and preventing the insider threat) presents the largest implementation challenge, in part because of the required processes for vetting employees for Biological Select Agents and Toxins.
    - The variability in implementation of security requirements across all research institutions presents a challenge when considering the effectiveness of federal governance. This variability results from differences in financial and human resources, lack of standards for security measures, institutional structure and support, and state, local, and institutional policies. The institutions that were visited as part of this effort differ significantly from those institutions that have been highlighted in the popular press as having poor security.
    - No set of best practices or validated practices exist for implementation of security requirements. Best or validated security measures could address concerns about variability in implementation of security requirements and about inconsistent inspections.
    - Security awareness appears to be high among administrators and employees who work with Biological Select Agents and Toxins and/or research involving animals. However, this awareness is not necessarily pervasive across the entire life science research community.
    - Some security measures, such as personnel security, are governed by other regulations, such as for restricting access to radioisotopes and certain hazardous chemicals. Some life science researchers are required to undergo personnel security assessments for work with radiological materials, chemicals, and Biological Select Agents and Toxins. However, each requirement and vetting process differs across the regulations and some processes are viewed are more effective than others.
    - One institution suggested the creation of an institutional mechanism through which the Responsible Official and campus police could share information about potential concerns, complaints, or arrests of Federal Select Agent Program-approved individuals.
    - In light of increasing cyber breaches in many sectors, innovative technical and policy options are lacking for securing computer systems that control facility operations and store or house data (e.g., surveillance footage, digital inventories, and personnel information) from hacking.
    - One of the more significant challenges is keeping pace with the changing social environment of US research laboratories. Though not evaluated sufficiently in this effort, the increasingly

multidisciplinary, multi-sectoral, international, and digital research enterprise likely will outpace conventional physical, electronic, and personnel security measures. However, development, validation, and adoption of security measures that both counters emerging threats *and* enable continued growth of this enterprise has yet to be addressed, and

- The reality that the statutory landscape governing Biological Select Agents and Toxins undergoes constant change presents difficulties to implementation of effective security measures, not simply measures to meet compliance requirements. Biosecurity regulations have become a moving target causing institutions difficulty in implementing the new requirements before the regulations change again.

- Biosafety measures for restricting personnel access to high containment laboratories, imposing physical and electronic barriers to restrict unauthorized access and preventing accidental release of the pathogen, and surveillance and monitoring have a dual purpose of enhancing safety and contributing to security.

- Of the institutions project staff visited, all implemented measures that either met or exceeded the federal requirements for security based on evaluating interview responses, measures observed on site, and compliance with federal requirements.

- Research administrators, and some senior scientists, have open and cooperative relationships with their institutional police and local FBI WMD Coordinator.

- The intense focus of security on a Biological Select Agents and Toxins results in missed opportunities for raising awareness of security risks across the entire life science research enterprise, and

- Significant issues remain, including the availability of adequate resources, consistency and clarity of security requirements and inspections, and classification nomenclature of pathogen categories.

16.16.12 Knowledge Gaps

In evaluating the security measures required for and implemented at research institutions conducting research with influenza, SARS-CoV, and MERS-CoV, several knowledge gaps emerged. Addressing these gaps may enable more comprehensive assessment of the security risks associated with the conduct of different types of pathogens. However, some of these gaps present a security risk if communicated in publicly available literature.

Knowledge gaps include:

- Security measures implemented at non-select agent, non-animal research facilities.

- The financial, human, educational, scientific, and security costs involved in implementing security requirements.

- The existence of standards for training inspectors who assess compliance with security requirements.

- Best practices for implementing security measures.

- The prevalence of additional physical security measures across all US BSL-3 laboratories not working with Select Agents is a knowledge gap. In part this is because the *NIH Physical Security Design Requirements* only applies to new or refurbished facilities, and the available information is insufficient to: a) judge how many BSL-3 laboratories are old and have not been refurbished, and, hence, are not required to meet the NIH physical security design requirements, and b) determine the difference in physical security between any such old laboratories and laboratories meeting the non-public NIH physical security design requirements, and

- Insufficient knowledge regarding the cumulative access delay for physical access barriers for non-BSAT and BSAT laboratories.

In October 2015, the US Government released recommendations by the Federal Experts Security Advisory Panel (FESAP) and the Fast Track Action Committee on Select Agent Regulations (FTAC-SAR) to strengthen biosafety and biosecurity practices and oversight of facilities that conduct BSAT research.[3014,3015,3016] These recommendations span from promoting an environment of security awareness to establishing a mechanism through which best practices can be shared. Some of these recommendations address long-time challenges of the regulated community, including some highlighted in this report, while others incorporate approaches taken by US Government agencies as part of their outreach activities.

Following a 90-day internal review of the Centers for Disease Control and Prevention (CDC)/ Division of Select Agents and Toxins, the CDC issued a report detailing specific recommendations for addressing the reviewers' observations on inspections, incident reporting, and transparency and public understanding.[3017] The CDC's observations are consistent with the challenges described in this report and previously highlighted by regulated research institutions.

[3014] U.S. Government. Fact Sheet: Enhancing Biosafety and Biosecurity. October 2015.
[3015] U.S. Government. Report of the Federal Experts Security Advisory Panel. December 2014.
[3016] National Science and Technology Council, Committee on Homeland and National Security. Fast Track Action Committee Report: Recommendations on the Select Agent Regulations Based on Broad Stakeholder Engagement. October 2015.
[3017] Centers for Disease Control and Prevention. CDC 90 Day Internal Review of the Division of Select Agents and Toxins. Accessible at: http://www.cdc.gov/phpr/dsat/full-report.htm. Accessed on November 5, 2015.

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WHITE PAPER

Gain-of-Function Research: Ethical Analysis

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Abstract Gain-of-function (GOF) research involves experimentation that aims or is expected to (and/or, perhaps, actually does) increase the transmissibility and/or virulence of pathogens. Such research, when conducted by responsible scientists, usually aims to improve understanding of disease causing agents, their interaction with human hosts, and/or their potential to cause pandemics. The ultimate objective of such research is to better inform public health and preparedness efforts and/or development of medical countermeasures. Despite these important potential benefits, GOF research (GOFR) can pose risks regarding biosecurity and biosafety. In 2014 the administration of US President Barack Obama called for a "pause" on funding (and relevant research with existing US Government funding) of GOF experiments involving influenza, SARS, and MERS viruses in particular. With announcement of this pause, the US Government launched a "deliberative process" regarding risks and benefits of GOFR to inform future funding decisions—and the US National Science Advisory Board for Biosecurity (NSABB) was tasked with making recommendations to the US Government on this matter. As part of this deliberative process the National Institutes of Health commissioned this Ethical Analysis White Paper, requesting that it provide (1) review and summary of ethical literature on GOFR, (2) identification and analysis of existing ethical and decision-making frameworks relevant to (i) the evaluation of risks and benefits of GOFR, (ii) decision-making about the conduct of GOF studies, and (iii) the development of US policy regarding GOFR (especially with respect to funding of GOFR), and (3) development of an ethical and decision-making framework that may be considered by NSABB when analyzing information provided by GOFR risk-benefit assessment, and when crafting its final recommendations (especially regarding policy decisions about funding of GOFR in

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particular). The ethical and decision-making framework ultimately developed is based on the idea that there are numerous ethically relevant dimensions upon which any given case of GOFR can fare better or worse (as opposed to there being necessary conditions that are either satisfied or not satisfied, where all must be satisfied in order for a given case of GOFR to be considered ethically acceptable): research imperative, proportionality, minimization of risks, manageability of risks, justice, good governance (i.e., democracy), evidence, and international outlook and engagement. Rather than drawing a sharp bright line between GOFR studies that are ethically acceptable and those that are ethically unacceptable, this framework is designed to indicate where any given study would fall on an ethical spectrum—where imaginable cases of GOFR might range from those that are most ethically acceptable (perhaps even ethically praiseworthy or ethically obligatory), at one end of the spectrum, to those that are most ethically problematic or unacceptable (and thus should not be funded, or conducted), at the other. The aim should be that any GOFR pursued (and/or funded) should be as far as possible towards the former end of the spectrum.

Keywords Gain-of-function research · Dual-use research · Biosafety · Biosecurity · Risk-benefit assessment · Decision theory

Executive Summary

Gain-of-function (GOF) research involves experimentation that aims or is expected to (and/or, perhaps, actually does) increase the transmissibility and/or virulence of pathogens. Such research, when conducted by responsible scientists, usually aims to improve understanding of disease causing agents, their interaction with human hosts, and/or their potential to cause pandemics. The ultimate objective of such research is to better inform public health and preparedness efforts and/or development of medical countermeasures. Despite these important potential benefits, GOF research (GOFR) can pose risks regarding biosecurity and biosafety. GOFR is a subset of "dual-use research"—i.e., research that can be used for both beneficial and malevolent purposes. Whereas the dual-use life science research debate has largely focused on biosecurity dangers associated with potential malevolent use of research, the GOFR debate has more explicitly focused on risks involving both biosecurity and biosafety—the point being that creation of especially dangerous pathogens might pose highly significant biosafety risks that are independent of, and perhaps more feasible to measure/assess than, risks associated with malevolent use.

Following controversy surrounding research, published in 2012, that led to the creation of highly pathogenic H5N1 (avian) influenza virus strains that were airborne transmissible between ferrets—and more recent reports of biosafety mishaps involving anthrax, smallpox, and H5N1 in government laboratories—in 2014 the administration of US President Barack Obama called for a "pause" on funding (and relevant research with existing US Government funding) of GOF experiments involving influenza, SARS, and MERS viruses in particular. This pause

applies specifically to experiments that "may be reasonably anticipated to confer attributes … such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route" (White House 2014). With announcement of this pause, the US Government launched a "deliberative process … to address key questions about the risks and benefits of gain-of-function studies" (White House 2014) to inform future funding decisions—and the National Science Advisory Board for Biosecurity (NSABB) was tasked with making recommendations to the US Government on this matter. As part of this deliberative process, the National Institutes of Health (NIH) commissioned this Ethical Analysis White Paper providing:

1. Review and summary of ethical literature on GOFR;
2. Identification and analysis of existing ethical and decision-making frameworks relevant to (i) the evaluation of risks and benefits of GOFR, (ii) decision-making about the conduct of GOF studies, and (iii) the development of US policy regarding GOFR (especially with respect to funding of GOFR); and
3. Development of an ethical and decision-making framework that may be considered by NSABB when analyzing information provided by GOFR risk-benefit assessment, and when crafting its final recommendations (especially regarding GOFR funding policy decisions in particular).

The ethical literature (discussed below) on GOFR to date has primarily focused on

- Biosafety concerns—e.g., that a devastating pandemic could potentially result from a laboratory accident involving an especially dangerous pathogen created via GOFR
- The need for objective risk-benefit analysis, broader community engagement/consultation, and more transparent GOFR decision- and policy-making
- The need to minimize risks—and controversy surrounding the nature and magnitude of likely risks of GOFR
- The requirement that research benefits outweigh risks—and controversy surrounding the nature and magnitude of likely benefits of GOFR

Following (1) discussion of the limitations of risk-benefit assessment as a guide to decision- and policy-making and (2) identification of numerous existing ethical and decision-making frameworks, and analysis of their general strengths and weaknesses and/or specific applicability to GOFR, this White Paper ultimately develops/proposes a framework for GOFR decision-and policy-making (especially regarding funding of GOFR) comprised of the following principles:

1. *Research Imperative* The ethical acceptability of GOFR posing extraordinary risks partly depends on the importance of the research question it aims to address.
2. *Proportionality* The ethical acceptability of extraordinarily risky GOFR partly depends on the extent to which there is reasonable expectation that the research

in question will (1) yield answers to the target public health question and (2) ultimately result in benefits that outweigh risks involved.

3. *Minimization of Risks* Other things being equal, the ethical acceptability of a GOFR study is a function of the degree to which (1) there is confidence that no less risky forms of research would be equally beneficial and (2) reasonable steps have been made to minimize risks of the GOFR study in question.
4. *Manageability of Risks* Other things being equal, the more manageable the risks of a GOFR study, the more ethically acceptable the study would be. Conversely, the more important/beneficial a GOFR study is expected to be, the more we should be willing to accept potentially unmanageable risks.
5. *Justice* Because justice requires fair sharing of benefits and burdens, the ethical acceptability of GOFR partly depends on the degree to which (1) risks fall on some people more than others, (2) risks fall on those who are unlikely to benefit, and/or (3) any resulting harms are uncompensated.
6. *Good Governance—Democracy* GOFR decision- and policy-making should (insofar as possible) reflect the ultimate values, value weightings, and risk-taking strategies of public citizens.
7. *Evidence* Decision- and policy-making regarding GOFR should be based on more/better evidence regarding risks, benefits, (means of) risk minimization, who is likely to benefit or be harmed by research, and the values, value weightings, and risk-taking strategies of public citizens.
8. *International Outlook and Engagement* Because risks and benefits of GOFR (can) affect the global community at large, the ethical acceptability of GOFR partly depends on the extent to which it is accepted internationally. Decision- and policy-making regarding GOFR should (insofar as possible) involve consultation, negotiation, coordination, and related forms of active engagement with other countries.

This framework is based on the idea that there are numerous ethically relevant dimensions upon which any given case of GOFR can fare better or worse (as opposed to there being necessary conditions that are either satisfied or not satisfied, where all must be satisfied in order for a given case of GOFR to be considered ethically acceptable). Rather than drawing a sharp bright line between GOFR studies that are ethically acceptable and those that are ethically unacceptable, this framework is designed to indicate where any given study would fall on an ethical spectrum—where imaginable cases of GOFR might range from those that are most ethically acceptable (perhaps even ethically praiseworthy or ethically obligatory) (i.e., those that fare best with respect to all 8 dimensions), at one end of the spectrum, to those that are most ethically problematic or unacceptable (i.e., those that fare worst regarding all 8 dimensions, and thus clearly should not be funded/conducted), at the other. The aim should be that any GOFR pursued (and/or funded) should be as far as possible towards the former end of the spectrum.

Introduction

Gain-of-function (GOF) research involves experimentation that aims or is expected to (and/or, perhaps, actually does) increase the transmissibility and/or virulence of pathogens. Such research, when conducted by responsible scientists, usually aims to improve understanding of disease causing agents, their interaction with human hosts, and/or their potential to cause pandemics. The ultimate objective of such research is to better inform public health and preparedness efforts and/or development of medical countermeasures. Despite these important potential benefits, GOF research (GOFR) can pose risks regarding biosecurity and biosafety.

GOFR is a subset of "dual-use research"—i.e., research that can be used for both beneficial and malevolent purposes (Miller and Selgelid 2008; National Research Council 2004). 'Dual-use research of concern' (DURC) refers to dual-use research for which the consequences of malevolent use would be exceptionally severe (whereas almost any research might be considered "dual-use" broadly conceived—because almost any research, or just about anything for that matter, can be used for some malevolent purpose or other). Of particular concern in the context of life science research is that advances in biotechnology may enable development and use of a new generation of biological weapons of mass destruction.

DURC has thus been one of the most hotly debated science policy issues during the 21st century, with controversy surrounding a series of published experiments with potential implications for biological weapons-making. Such studies include the genetic engineering of a superstrain of the mousepox virus in 2001 (Jackson et al. 2001), the artificial synthesis (via synthetic genomics) of a "live" polio virus from chemical components in 2002 (Cello et al. 2002), and the reconstruction (via synthetic genomics) of the 1918 "Spanish Flu" virus in 2005 (Tumpey et al. 2005). Though all of these studies involved legitimate aims, critics argued that they should not have been conducted and/or published. Some argued that publishing studies like these in full detail provided "recipes" for especially dangerous potential biological weapons agents to would-be bioterrorists. Many who acknowledged such potential dangers, on the other hand, argued that benefits of publication outweighed risks involved.

The most controversial dual-use life science experiments to date involved the creation of highly pathogenic H5N1 (avian) influenza virus strains that were airborne transmissible between ferrets, which provide the best model for influenza in humans (Herfst et al. 2012; Imai et al. 2012). This research addressed an important scientific question—i.e., Might it be possible for H5N1 to naturally evolve into a human-to-human transmissible strain and thus result in a pandemic?—and (purportedly) yielded an affirmative answer. After the US National Science Advisory Board for Biosecurity (NSABB) initially recommended that these studies should be published in a redacted form (i.e., including key findings, while omitting detailed description of materials and methods), it later approved publication of revised versions in full, and the papers were published in 2012. Advocates of these studies/publications argued that they would improve surveillance of H5N1 in nature (facilitating early identification of, and thus better response to, the emergence of

potential pandemic strains) and facilitate development of vaccines that might be needed to protect against pandemic strains of the virus. Critics questioned the validity of claims about such benefits and argued that the studies might facilitate creation of biological weapons agents that could kill millions, or possibly even billions, of people.

While the concern about the biological weapons implications of this ferret H5N1 research pertains to dangers of dual-use life science research as traditionally conceived, many of the objections to this research additionally addressed the danger that the pathogens created might have escaped from laboratories, and potential consequences thereof—and there were particular concerns about the conditions under which this research was conducted (e.g., the safety level of the laboratories where this research was conducted). Controversy surrounding these ferret H5N1 experiments has thus lead to a significant shift in debate about dual-use research to framing in terms of "gain-of-function research". Whereas the dual-use debate largely focused on biosecurity dangers associated with potential malevolent use of research, the GOFR debate has more explicitly focused on risks involving *both biosecurity and biosafety*—the point being that creation of especially dangerous pathogens might pose highly significant biosafety risks that are independent of, and perhaps more feasible to measure/assess than, risks associated with malevolent use.

Since the first high-profile DURC life science experiments were published in the early 2000s, much policy debate has surrounded questions about how DURC should be governed. Among other things, it has been argued that increased oversight of research and/or publication of potentially dangerous discoveries may be necessary, that codes of conduct for scientists (explicitly addressing dual use issues) should be adopted, and/or that scientists should be further educated about the dual use phenomenon and ethics; and relevant policies have been implemented to varying degrees in different countries. In light of the ferret H5N1 research controversy, furthermore, influenza researchers imposed a voluntary moratorium on GOF studies from January 2012 to February 2013; and the US Government developed/adopted policy regarding the funding of GOF H5N1 studies in 2013 (Department of Health and Human Services 2013).

Following more recent reports of biosafety mishaps involving anthrax, smallpox, and H5N1 in government laboratories—and burgeoning debate regarding biosafety risks of GOFR more generally (Kaiser 2014)—in 2014 the administration of US President Barack Obama called for a "pause" on funding (and relevant research with existing US Government funding) of GOF experiments involving influenza, SARS, and MERS viruses in particular. This pause applies specifically to experiments that "may be reasonably anticipated to confer attributes … such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route" (White House 2014). With announcement of this pause, the US Government launched a "deliberative process … to address key questions about the risks and benefits of gain-of-function studies" (White House 2014) to inform future funding decisions—and NSABB was tasked with making recommendations to the US Government on this matter. As part of this deliberative process, the National Institutes of Health (NIH) commissioned this Ethical Analysis White Paper providing:

1. Review and summary of ethical literature on GOFR;
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3. Development of an ethical and decision-making framework that may be considered by NSABB when analyzing information provided by GOFR risk-benefit assessment, and when crafting its final recommendations (especially regarding GOFR funding policy decisions in particular).

Gain-of-Function Research Ethics: State of Debate

Just as (bio)ethicists were slow to engage in debate about dual-use life science research more generally (Selgelid 2010), it is noteworthy that (with very few exceptions) most of the existing literature explicitly addressing gain-of-function research (i.e., using the language of 'gain-of-function research') has not been authored by (bio)ethicists in particular. Even when authored by scholars from other disciplines, furthermore, most of the existing ethically relevant GOFR literature is neither explicitly focused on ethics (e.g., using the language of 'ethics' in titles, abstracts, or key words) nor published in (bio)ethics journals. On the other hand, much of the literature surrounding GOFR controversy is (largely) implicitly concerned with ethics (whether or not the language of 'ethics' is front and center) insofar as normative considerations, values, questions about how to weigh risks/harms against benefits, and questions about "what ought to be done"—all of which fall squarely within the domain of ethics—are of central concern. The following thus aims to summarize the main ethical issues/points raised in literature explicitly concerned with GOFR (using the language of 'gain-of-function research') whether or not the papers in question were authored by ethicists, published in ethics journals, or explicitly employ widespread use of the language of 'ethics'.[1]

Biocontainment

A distinct aspect of the shift in debate from framing in terms of "dual-use research" to "gain-of-function research" has been focus on biosafety concerns—e.g., that a

[1] Whether or not ethics is their explicit main focus, most papers covered in this literature review were identified because they at least mention both 'ethics' and 'gain-of-function research' and thus turned up in systematic literature searches of relevant databases. Due to space constraints, the following does not aim to comprehensively cover literature concerned with dual-use life science research more generally—or even the controversial ferret H5N1 studies mentioned above, unless 'gain-of-function research' is explicitly mentioned—except insofar as the papers in question are directly relevant to risk-benefit analysis and/or the ethical- and decision-making framework parts of this paper. Much of the explicitly ethical literature concerned with dual-use life science research more generally, including the ferret H5N1 studies (prior to framing in terms of 'gain-of-function research'), are largely concerned with the ethical responsibilities of scientists and/or issues of censorship which are beyond the scope of this paper, which focuses on the shift in debate (towards biosafety and risk-benefit analysis) that occurred with framing in terms of 'gain-of-function research'.

devastating pandemic could potentially result from a laboratory accident involving an especially dangerous pathogen created via GOFR. In light of Ron Fouchier's claim that the ferret-transmissible strain of H5N1 he produced is "probably one of the most dangerous viruses you can make" (Enserink 2011) and (previous) NSABB chair Paul Keim's claim that "I can't think of another pathogenic organism as scary as this one [created by Fouchier's team] … I don't think anthrax is scary at all compared to this" (Enserink 2011), for example, some critics argued that the study in question should have been, and/or that future similar research should be, conducted in laboratories with the highest bio-containment level—i.e., biosafety level 4 (BSL-4), as opposed to BSL-3 ("enhanced") in which this research was done (Swazo 2013). Fouchier has, in response, pointed out that his research received necessary institutional biosafety review/approval; and others have argued that his research (given employment of safety measures beyond ordinary BSL-3, including vaccination of lab workers against H5N1) in effect involved safety equivalent to BSL-4 (Roos 2012). Anthony Fauci (Director of the US National Institute of Allergy and Infectious Diseases) has concluded that "the scientists who triggered this debate [including Fouchier] … have conducted their research properly and under the safest and most secure conditions" (Fauci 2012, p. 1).

Additional biosafety concerns involve potential dangers associated with proliferation of GOFR, which is arguably likely to occur as more similar work is conducted/published. Whether or not GOFR has been adequately safe to date, similar future research might be conducted in suboptimal conditions—e.g., in countries/institutions with weaker infrastructure and/or research oversight systems (Evans et al. 2015; Fauci 2012; Gronvall 2014; Lipsitch and Galvani 2014; Wain-Hobson 2014). Part of the resistance to insistence that additional similar research be conducted in BSL-4, on the other hand, is that this might unnecessarily increase expense, reduce efficiency, and/or inequitably deem relevant research impermissible in less wealthy countries (Lipkin 2012).

Broad Community Engagement, Risk-Benefit Analysis, and Transparency

One clear consensus in (ethically relevant) literature addressing GOFR is that there is need for broader community engagement/consultation and more transparent decision- and policy-making (Duprex et al. 2015; Evans et al. 2015; Fauci 2012; Imperiale and Casadevall 2015; Lipsitch and Galvani 2014; Lipsitch and Inglesby 2014; Pfeiffer 2015; Suk et al. 2014). Part of the concern here, hopefully to be addressed by the deliberative process initiated by the US Government, is the perception (at least in the eyes of some) that much of the relevant debate and/or decision-making to date has been dominated by a limited subset of the scientific community and/or by people or parties with potential conflicts of interest. Because the potential risks and benefits of GOFR affect the public at large, it has been argued that more public input to debate and decision-making is necessary—the idea being that it is ethically problematic for some (e.g., scientists) to be making decisions and taking actions that impose serious risks on others (i.e., members of the general public) without consent of, or adequate input from, the latter. Furthermore, because the consequences of GOFR are ultimately global in nature (i.e., GOFR conducted in

one country can have risks and benefits for those living in other countries), many have emphasized the importance of greater international engagement, which is necessary to promote harmonization of GOFR governance. While it is widely accepted that expert scientific opinion is essential to well-informed GOFR decision- and policy-making, there have been calls for input from a wider range of scientific disciplines. Jonathan Suk and colleagues (2014), for example, argue that greater engagement with public health experts would facilitate both (1) assessment of GOFR risks and benefits and (2) design of GOFR studies that would have better translation into public health policy and practice. Many of these points are captured by the following statement of David Relman:

> Woefully insufficient input has been obtained from a wide variety of scientists and from many other stakeholders among the general public. It is unethical to place so many members of the public at risk and then consult only scientists—or, even worse, just a small subset of scientists—and exclude others from the decision-making and oversight process … In many cases, conversations have only involved infectious-disease researchers and conflicts of interests among participants have not been adequately acknowledged or addressed … It is our responsibility as scientists to explain the rationale behind our work, including its benefits and risks, to the general public in terms that are accessible to those with an average level of education, rather than to be dismissive. This is especially important when the work has important consequences for the whole of society (Relman in Duprex et al. 2015, pp. 61–63).

There has likewise been broad support for the conduct—and transparent public dissemination—of GOFR risk-benefit analysis. Advocacy for risk-benefit analysis is partly motivated by recognition that any policy judgment that the benefits of any given GOFR study outweigh the risks (or vice-versa) should, insofar as possible, be evidence-based—and transparency is important because members of the public expect (and deserve) to be informed about the bases upon which key judgments/decisions are made (Fauci 2012).

While Kirsten Jacobson and colleagues suggest that, in light of measurement difficulties, "[a] qualitative risk-benefit analysis framework for assessing research…would be the most decisive tool for asking the hardest and most important questions" (Jacobson et al. 2014, p. 3), Marc Lipsitch and Thomas Inglesby argue that risk-benefit analysis can and should be quantitative because "[e]xtensive qualitative arguments have not provided sufficient clarity or evidence to resolve concerns or identify a consensus path forward … this process should be quantitative, rather than relying on unquantified and unverifiable assurances that particular laboratories are safe" (Lipsitch and Inglesby 2014, pp. 1, 5). Though they admit measurement challenges associated with objective quantitative risk-benefit analysis, Arturo Casadevall and Michael Imperiale (Casadevall and Imperiale 2014; Imperiale and Casadevall 2015) nevertheless argue that performing such analysis with the best available evidence could at least facilitate experimental designs that reduce risks or enhance benefits.

Risk Measurement and Minimization

While it has long been acknowledged that biosecurity risks associated with dual-use life science research are especially difficult (if not impossible) to estimate with confidence (e.g., given unpredictable actions of potential malevolent actors) (Posner 2004), Lipsitch and Inglesby (2014) argue that the historical record of laboratory accidents at least enables evidence-based quantitative assessment of GOFR biosafety risks in particular. As summarized by Daniel Rozell (2015, p. 1), however, early attempts at quantitative GOFR risk assessment have lead to widely divergent estimations:

> Using biosafety level 3 (BSL-3) lab infection data, Lipsitch and Inglesby [2014] estimated a probability of between 0.01 % and 0.1 % per laboratory-year of creating a pandemic which would cause between 2 million and 1.4 billion fatalities. This yields an expected fatality rate of 2000 to 1.4 million per BSL-3 laboratory-year. Alternatively, using data from the National Institutes of Allergy and Infectious Diseases, the probability of a pandemic would be between 0.05 % and 0.6 % per worker-year, with a resulting expected fatality rate of between 10,000 and 10 million per laboratory worker … A subsequent risk estimate from Fouchier [2015] started from the same data, but then Fouchier argued that [given special safety precautions taken in his H5N1 GOFR] a lab-induced pandemic would occur every 33 billion years—more than twice the known age of the universe.

Though further details of such calculations/analyses are beyond the scope of this paper, the risk-benefit assessment commissioned by the US Government will hopefully help resolve this controversy. In the meanwhile, even if Fouchier's estimates about his own research are correct, which Lipsitch and Inglesby (2015) dispute, concerns about proliferation of GOFR conducted in less safe conditions should not be forgotten.

Despite this debate regarding the magnitude of biosafety risks posed by GOFR, there appears to be fairly widespread agreement that, other things being equal, research risks should be minimized (Casadevall et al. 2014a, b; Casadevall and Imperiale 2014; DHHS 2013; Duprex et al. 2015; Evans et al. 2015; Imperiale and Casadevall 2015; Lipsitch and Galvani 2014; Lipsitch and Inglesby 2014). It has been suggested that GOFR risks might be reduced via:

- Employment of safer pathogen strains
  - of low virulence,
  - for which there is immunity,
  - for which there are existing vaccines, and/or
  - which have been modified to inhibit replication outside of laboratories;
- Development/use of vaccines against experimental pathogen strains;
- Development/use of broad spectrum vaccines (e.g., pan- or universal influenza vaccine);
- Vaccination of laboratory workers to create a ring of immunity; and/or

- Ongoing improvement of biosafety practice and infrastructure.

Alternatively, it has been argued that research risks should be minimized via conduct of other less risky kinds of research rather than GOFR—at least in cases where the former would be equally beneficial in answering key scientific questions and/or achieving public health goals (see Lipsitch comments in Duprex et al. 2015; Evans et al. 2015; Lipsitch and Galvani 2014; Lipsitch and Inglesby 2014).

Benefit Controversy

While the decision to publish the initial ferret H5N1 influenza studies of the research teams headed by Ron Fouchier and Yoshihiro Kawaoka (Herfst et al. 2012; Imai et al. 2012) in full was based on the judgment that benefits of publication outweighed the risks, numerous critics have questioned the actual benefits of these studies. Purported benefits of publication were that this would facilitate (1) development/production of vaccines against pandemic strains of the virus and (2) surveillance enabling early identification of, and thus response to, pandemic strains that might occur naturally. Critics have argued that such benefits are limited, *inter alia*, because naturally occurring pandemic strains may turn out be different from those created via the studies in question (in which case production of vaccines for, or surveillance targeting of, the latter might not be very useful); international surveillance systems are too weak "to detect a pandemic viral sequence … before it is too late" (Lipsitch and Galvani 2014, p. 3); "an important lesson learnt from pandemic H1N1 (swine flu) is that there is not much that can be done to contain outbreaks of pandemic strains of influenza once they emerge" (i.e., so early identification via surveillance might not make much difference) (Selgelid 2013, p. 148); and, given the way the vaccine industry actually works, there is unlikely to be development/stockpiling of vaccines against naturally-occurring transmissible strains of influenza before such strains actually arise (Selgelid 2013).

Lipsitch and Alison Galvani (2014) have additionally disputed the suggestion that these studies answered important public health questions—i.e., whether H5N1 might mutate into a human transmissible strain and what kinds of mutations might make this possible—in light of general difficulties translating ferret findings to humans (i.e., we cannot be sure that a strain of influenza transmissible in ferrets would be transmissible in humans) and complexities regarding epistasis (i.e., the phenotypic effects of any given mutation may depend on the broader genetic background of the organism in question; the same mutation may have different effects in different strains of a pathogen). In response to the point about translatability of ferret research to humans, Imperiale and Casadevall (2015) have responded that if this is a reason to be skeptical about benefits then it is also a reason to be skeptical about risks associated with the research in question.

While the reality or magnitude of risks associated with dual-use and GOF research have frequently been questioned (e.g., is malevolent use a tangible/significant threat or merely a theoretical possibility?), Nicholas Evans has argued that purported benefits of dual-use and GOF research should likewise not be simply taken for granted. Whether or not theoretically possible benefits of any given study

are realized, according to Evans, will depend on background institutional factors (e.g., strength of healthcare infrastructure(s), systems of surveillance and countermeasure production, political will and resources necessary to translate scientific findings into benefits) that may or may not exist and/or may vary widely from country to country (Evans 2013, 2014a, b; Evans et al. 2015).

This last point highlights justice implications of GOFR—i.e., because some (people or countries) will be better able to protect against risks and/or realize benefits from GOFR than others. Alta Charo, for example, argues that:

> the benefits [of GOFR] will disproportionately go to people who are either personally better off or in wealthier countries because that is often where the healthcare system or economic access to healthcare is better. We need to pay more attention to making sure that the benefits are justly distributed and the science is beneficial for everybody (NRC and IOM 2015, p. 66).

Casadevall and colleagues (2014a, b) emphasize potential epistemic benefits of GOFR. They argue that the controversial H5N1 ferret research, employing well-established scientific methodology, provided the only way to demonstrate with certainty the possibility that H5N1 "had the biological capacity to generate variants that could spread from mammal to mammal" (2014a, p. 2). Acknowledging that potential benefits of advances in scientific knowledge may be long term—and difficult to predict ahead of time—they nonetheless maintain that GOFR benefits in the way of knowledge production should be taken into consideration, and not underestimated, in risk-benefit analysis of GOFR. Evans (2014a) concurs that scientific knowledge is valuable, but argues that appropriately factoring scientific knowledge advancement into risk-benefit analysis requires clarity regarding whether or not, or the extent to which, knowledge should be considered intrinsically valuable (i.e., valuable for its own sake) as opposed to merely instrumentally valuable (i.e., valuable only insofar as it promotes realization of other things of intrinsic value).

Given the value of scientific knowledge advancement, numerous authors have warned about various ways in which GOFR controversy could stall important areas of scientific development (Casadevall and Imperiale 2014; Duprex et al. 2015; Evans et al. 2015; Fauci 2012; Imperiale and Casadevall 2015; Lipsitch and Inglesby 2014; Pfeiffer 2015; Suk et al. 2014; Wain-Hobson 2014). An untoward event could lead to societal backlash, for example, and/or increased regulations may discourage scientists from pursuing certain kinds of research. Such worries highlight one reason, among many, why good governance of GOFR is crucial.

Arguing that (1) numerous other kinds of scientific research and/or public health activities would be equally (or more) beneficial in answering key scientific questions and/or promoting public health goals and (2) GOFR creation of potential pandemic pathogens (PPPs) poses large risks to large numbers of people, Lipsitch and co-authors conclude that (3) the benefits of GOFR creation of PPPs do not outweigh the risks, and thus that GOFR creation of PPPs should be considered unjustified (unless, at least, objective quantitative risk-benefit analysis proves otherwise). In reaching this conclusion, they appeal to Nuremburg Code and Belmont Report requirements that research should "be done only if it benefits society, if the same benefits could not be procured through less risky means, and if the anticipated benefits exceed the

anticipated risk" (Evans et al. 2015). Though they acknowledge that the Nuremburg Code and Belmont Report were explicitly designed to govern research involving human subjects, they argue that (in light of the general ethical considerations upon which such guidelines are based) these requirements have broader applicability to risky research more generally. While Lipsitch and co-authors advocate quantitative GOFR risk-benefit analysis, they emphasize the importance of assessing GOFR studies "on the basis of their marginal benefits, compared to those of safer approaches" (the idea being that any increased risks must be outweighed by increased benefits in order for GOFR studies to be justified) (Lipsitch and Galvani 2014, p. 5).

Risk-Benefit Assessment of Gain-of-Function Research

As part of the deliberative process called for during the pause on selected gain-of-function research involving influenza, MERS, and SARS viruses, the US Government has commissioned an in-depth, systematic assessment of the risks and benefits specifically associated with this kind of research. In its *Framework for Conducting Risk and Benefit of Gain-of-Function Research*, NSABB (2015) has recommended that the contractor responsible for this work assess the following kinds of potential (possibly overlapping) risks and benefits, including security implications thereof, in particular:

Risks

- Biosafety—i.e. dangers associated with laboratory accidents;
- Biosecurity—i.e., dangers associated with crime and terrorism if pathogens are not physically secure and/or if malevolent actors gain access to them;
- Proliferation—i.e., dangers that might grow proportionally with an increased rate of GOFR, potentially in different settings with varying biosafety standards;
- Information risk—i.e., if published studies facilitate malevolent action (e.g., by terrorists) or, possibly, breach of intellectual property;
- Agricultural—i.e., risks to agriculturally-relevant animals if enhanced pathogens arising from GOFR are accidentally or intentionally released into animal populations, and possible implications for human health;
- Economic risks—i.e., financial implications of (accidental or intentional) pathogen release or, possibly, opportunity costs; and
- Loss of public confidence—i.e., compromise of trust in the scientific enterprise that could result from (accidental or intentional) pathogen release.

Benefits

- Scientific knowledge—i.e., (potentially unique) information gained, and the value of such information for understanding pathogens/disease;
- Biosurveillance—i.e., enhancement of (a) public health surveillance, (b) agricultural and domestic animal surveillance, and (c) wildlife surveillance—to improve outbreak detection/prediction and/or decision-making;

- Medical countermeasures—i.e., (potentially unique) information facilitating development of therapeutics, vaccines, and diagnostics;
- Informing policy decisions—i.e., regarding public health preparedness (e.g., countermeasure stockpiling, vaccine strain selection, resource mobilization); and
- Economic benefits—i.e., financial gains (e.g., from industrial productivity) and/or cost savings (e.g., from reduced health care expense).

The conduct and dissemination of findings from this risk-benefit assessment (RBA) will (1) address a demand expressed by commentators in debate surrounding GOFR (i.e., that RBA is conducted and made public), (2) hopefully help resolve controversy surrounding the extent of risks and/or benefits of GOFR (e.g., empirical debates about the magnitude of biosafety risks discussed in the above literature review), and (3) inform policy-making regarding the funding and conduct of GOFR.

Risk-Benefit Assessment Limitations

While the commissioned RBA will be valuable in all of these ways, it may be a mistake to think that RBA will provide a panacea for solving difficult policy issues surrounding GOFR. Though RBA could undoubtedly promote better informed policy decisions (and thus better policy decisions), for example, it is perhaps unlikely that RBA will itself provide a clear guide to action regarding the funding and conduct of GOFR.[2] This is for numerous reasons.

Complexity and Uncertainty

First, given the inordinate complexities involved with assessing the risks and benefits of GOFR—considering, for example, all the possible scenarios for better or worse that might arise, and the enormous number of factors that outcomes depend on—it would be difficult for RBA to reveal, with a high degree of confidence anyway, the likelihood and magnitude of harms and/or benefits that could result from GOFR. A widely acknowledged limitation of RBA is that confidence in predictions generated depends upon the quality of (1) input data and (2) models employed in assessment of risks and benefits.[3] Both data and models will inevitably be imperfect in the context of GOFR in light of scenario complexity, uncertainties, unknown unknowns, and presumably *unknowable* unknowns, that are relevant to GOFR consequences. The likelihood and magnitude of harms that could result from GOFR, for example, partly depend upon the actions of malevolent actors. There are

[2] This, of course, depends on the outcome of RBA. If, hypothetically, RBA demonstrated with a high degree of confidence that GOFR (or a certain case of GOFR, conducted under certain conditions) would be enormously beneficial in numerous ways without imposing major/significant risks—or if RBA demonstrated with a high degree of confidence that GOFR (or a certain case of GOFR, conducted under certain conditions) would be extremely risky in numerous ways without promoting major/significant benefits—then RBA might itself provide a clear guide to action. If things were this simple, however, GOFR would likely not be so controversial to begin with.

[3] For discussion of "model risk"—i.e. "the risk that the model is inappropriate for the problem"—and ways in which this may be addressed, see the work of Peter Taylor (2012).

innumerable possible actions that such actors might take, however—and the likelihood of any given action and/or the consequences thereof (given all the relevant factors involved) are arguably inestimable (Posner 2004). In some cases the commissioned RBA will aim to provide qualitative rather than quantitative analysis—precisely because the latter will not always be feasible. At the September 2015 meeting of the NSABB, Rocco Casagrande (Managing Director of Gryphon Scientific, which has been commissioned to complete the RBA currently underway) explained that assessment of potential benefits of GOFR (e.g., regarding countermeasure development) will be qualitative rather than quantitative because there is inadequate data for the latter (Casagrande 2015).[4]

RBA will, despite challenges noted above, hopefully provide the best assessments possible, acknowledging limitations regarding both quantitative and qualitative findings—and this would provide valuable input to decision-making processes. It is better to make informed rather than uninformed policy decisions regarding GOFR—and we can only inform ourselves to the best of our ability. To the degree that findings are uncertain (because based on imperfect data, estimates, and/or models), however, they may need to be considered with caution.

When Do Benefits Outweigh Risks and Vice Versa?

Quantification

Second, the findings of RBA might not themselves reveal whether expected benefits actually outweigh expected risks, or vice versa. This is partly because, as noted above, not all expected risks and benefits will be quantified by the RBA endeavor currently underway. Unless potential benefits of GOFR are quantified (e.g., in terms of the expected number of lives saved—given the likelihood and extent of life-saving that may result from potential improvement of countermeasures), it may not be obvious whether they outweigh quantified risks (e.g., in terms of expected number of lives lost—given the likelihood and severity of possible untoward outcomes resulting from GOFR).[5]

Values and Weightings

Even if all assessed risks and benefits were in fact quantified with a high degree of confidence, this may still not determine whether benefits outweigh risks, or vice versa, because that would depend on how benefits (or the ultimate values they promote) should be weighed against risks (or the ultimate values they compromise). *Inter alia*, this reveals the need for distinguishing things that are merely

[4] Given existing data regarding numbers of laboratory accidents and consequences thereof under various conditions (at least some) biosafety risks are being assessed quantitatively.

[5] This, again, depends on RBA findings. If GOFR is determined to be especially beneficial (qualitatively speaking with regard to countermeasure development and/or quantitatively with regard to other benefits, if any are actually quantified) with no major/significant risks, then it might be safe to conclude that benefits outweigh risks.

instrumentally valuable (i.e., valuable because they promote what is intrinsically valuable) from things that are intrinsically valuable (i.e., valuable for their own sake). Whether or not benefits outweigh risks, or vice versa, ultimately depends on whether there is (expected to be) net gain or loss of that which is intrinsically (or ultimately) valuable. To itself provide a clear guide to action, RBA would thus need to quantify or otherwise assess ultimate implications of GOFR regarding that which is intrinsically (or ultimately) valuable.

Many of the benefits and risks to be evaluated by RBA are presumably merely instrumentally valuable. Medical countermeasures, surveillance, and economic gains, for example, are arguably largely valuable not for their own sakes but in virtue of the role they play in protecting and/or promoting human well being (in the way of public health).[6] Presumably almost everyone will agree that human well being (in the way of public health) is one of the things that ultimately matters for its own sake, and thus one of the things that policy should ultimately aim to promote.[7]

The nature of other values associated with potential risks and benefits of GOFR, on the other hand, might not be so clear. There may be reasonable disagreement, for example, about whether the gain of scientific knowledge is merely instrumentally valuable, or also valuable for its own sake (Kitcher 2001; Evans 2014a). Similar things might be said about the value of security, which looms large in debates about GOF research. Policy debates about dual use research more generally have often been framed in terms of potential conflict, and/or the need to strike a balance, between the value of security, on the one hand, and the value of scientific progress, and the good things thereby enabled, on the other. In its *Framework for Conducting Risk and Benefit of Gain-of-Function Research* NSABB (2015) has recommended that the RBA contractor consider the security implications of the kinds of risks and benefits enumerated above. Conceived as the "protection of valuable things against loss" (Selgelid 2012), security can be considered a meta-value. Protection of valuable things against loss can include both protection of instrumentally valuable things against loss and protection of intrinsically valuable things against loss. In the latter case, the value of security pertains to the good of society writ large. Among other things, the ultimate good of society arguably consists in (aggregate) human well being, liberty, equality, and our democratic way of life. All of these values could potentially be compromised by pandemic risks that GOFR might reduce or exacerbate. While security (conceived as protection of such things against loss) is thus especially important, there might be reasonable disagreement about whether or not, or the extent to which, security is intrinsically valuable, or merely valuable insofar as it plays a role in promoting such things. This is an important (rather than merely academic) matter because it raises the question of whether or not, or the

[6] With regard to economic benefits, money is a prototypical example of a merely instrumentally valuable good. Given complexities involved with GOFR RBA, it might be reasonable to consider number of lives saved or lost as a (simplifying) proxy measure for human well being (or public health) impact. Another possibility would be to quantify possible well being gains or losses in terms of DALYs (i.e., disability adjusted life years lost—which is a common measure of burden of disease).

[7] According to utilitarian ethical theory well being is the only thing that is intrinsically valuable, and thus what policy should ultimately aim to maximize.

extent to which, it would be legitimate to make net sacrifices of (other things of) intrinsic value in order to gain more security.[8]

Part of the purpose of the discussion above is to reveal complexity surrounding the anatomy of values, and the importance of clarity regarding value hierarchy. Determining whether benefits of GOFR outweigh risks requires (1) distinguishing things that are intrinsically valuable from those that are merely instrumentally valuable and (then) (2) determining whether GOFR (or any particular case of GOFR) would lead to net benefit regarding the former kinds of goods in particular. RBA, however, will not settle questions about which goods pertaining to risks and benefits of GOFR are intrinsically valuable, because this is a matter of ethics rather than empirical science.

Even if a list of intrinsically valuable goods were taken as given, additional difficult ethical questions arise. First is the question of how potentially conflicting intrinsic goods should be weighed against one another—e.g., if GOFR would promote net gains in terms of some (e.g., aggregate well being) at net cost in terms of others (e.g., individual liberty in the way of freedom from significant risks in the absence of consent). Second is the question of the weight that should be given to benefits that may arise in the future—i.e., what, if anything, should the "future discount rate" be in the event that GOFR entails significant risks at present in order to achieve net benefits in the future (and/or for future generations) (Murray 1994). Third, and especially important, is the question of risk aversion, risk appetite, and/or risk-taking strategy. It is common to place greater disvalue on losses than value on gains (e.g., in things like well being or money) of equal magnitude, and it is not obviously irrational to do so. Whether or not benefits of GOFR are thought to outweigh risks may thus (depending on RBA findings) partly depend on what is considered appropriate risk-taking strategy (e.g., to what extent, if any, should decision-making reflect risk aversion?). Different risk-taking strategies embodying different levels of risk aversion may yield different answers to questions about what should be done if RBA reveals that GOFR (or a certain case thereof) is reasonably likely to promote a significant amount of human well being (e.g., by facilitating disease control) but has a very small chance of leading to catastrophic consequences (e.g., in the event of laboratory accident or malevolent use of research findings).

Existing Ethical and Decision-Making Frameworks

The above-mentioned limitations of RBA highlight the importance of ethical input to decision- and policy-making regarding the funding and conduct of GOFR. Such decision- and policy-making ultimately concerns questions about what should (or ought to) be done in light of information provided by RBA; and questions about what should (or ought to) be done is, by definition, what the discipline of ethics aims to address. This section outlines a variety of existing ethical and decision-making

[8] For related discussion of the value of security, and its relevance to health policy-making, see Jonathan Herington (2016).

frameworks that might be brought to bear on decision- and policy-making regarding GOFR.

Decision Theory

Expected Utility Maximization

A well-developed, and much discussed, approach to decision-making in contexts of risk holds that it would be rational to choose the action (or policy) with *maximum expected utility*, where the expected utility of any given action (or policy) is defined as the sum of the products of the likelihood and utility (or value) of each possible outcome of that action (or policy). Suppose, for example, that there are two options with the following possible consequences:

- Option A, which has two possible outcomes:
  - There is a 50 % (or .5) chance that Option A will lead to outcome A1, which embodies UA1 amount of utility (or value).
  - There is a 50 % (or .5) chance that Option A will lead to outcome A2, which embodies UA2 amount of utility (or value).
- Option B, which has 3 possible outcomes:
  - There is a 60 % (or .6) chance that Option B will lead to outcome B1, which embodies UB1 amount of utility (or value).
  - There is a 30 % (or .3) chance that Option B will lead to outcome B2, which embodies UB2 amount of utility (or value).
  - There is a 10 % (or .1) chance that Option B will lead to outcome B3, which embodies UB3 amount of utility (or value).

The expected utility of Option A (EUA) and the expected utility of Option B (EUB) would be calculated as follows:

$$\mathrm{EUA} = (.5 \times \mathrm{UA1}) + (.5 \times \mathrm{UA2})$$
$$\mathrm{EUB} = (.6 \times \mathrm{UB1}) + (.3 \times \mathrm{UB2}) + (.1 \times \mathrm{UB3})$$

According to the expected utility maximization approach to decision-making, it would be rational to choose Option A if EUA is greater than EUB; and it would be rational to choose Option B if EUB is greater than EUA.

Suppose, hypothetically, that RBA findings regarding risks and benefits of (a particular case of) GOFR involving H5N1 avian influenza virus reveal that we are ultimately faced with the following choice situation[9]:

[9] Related/similar illustrations of this kind of approach to decision-making are provided by Thomas Douglas (2013) and David Resnik (2014). The example provided here is, for reasons discussed above and below, an over simplification of what actual choice situations regarding GOFR would be like; and the numbers used are not assumed to accurate or realistic.

- Option 1: Refrain from GOFR, which would entail the following possible outcomes:
  - There is a 10 % (.1) chance that H5N1 naturally mutates into a pandemic strain that kills 100,000,000 people (in the absence of improved control measures that might have been possible via GOFR).
  - There is a 90 % (.9) chance that no H5N1 pandemic occurs, so no lives are lost.

- Option 2: Pursue GOFR, which would entail the following possible outcomes:
  - There is a 5 % (.05) chance that H5N1 naturally mutates into a pandemic strain that kills 100,000,000 people (because GOFR does not lead to improved control measures).
  - There is a 5 % (.05) chance that H5N1 naturally mutates into a pandemic strain that kills only 40,000,000 people (because GOFR results in effective new control measures).
  - There is a .6 % (.006) chance that laboratory accident or malevolent action leads to an H5N1 pandemic (involving a strain that might have occurred naturally) killing 100,000,000 people (because GOFR has not, or not yet, lead to effective new control measures).
  - There is a .4 % (.004) chance that laboratory accident or malevolent action leads to an H5N1 pandemic (involving a strain that might have occurred naturally) killing only 40,000,000 people (because GOFR results in effective new control measures).
  - There is a .06 % (.0006) chance that laboratory accident or malevolent action leads to an H5N1 pandemic (involving a strain more dangerous than would have arisen naturally) killing 2,500,000,000 people (because GOFR has not, or not yet, lead to effective new control measures).
  - There is a .04 % (.0004) chance that laboratory accident or malevolent action leads to an H5N1 pandemic (involving a strain more dangerous than would have arisen naturally) killing (only!) 1,000,000,000 people (because GOFR results in effective new control measures).
  - There is an 88.9 % (.889) chance that no H5N1 pandemic occurs, so no lives are lost.

Assuming that utility/value is determined by number of lives lost, then the expected utility of Option 1 (i.e., refraining from GOFR) would be:

$$(.1 \times 100{,}000{,}000 \text{ lives lost}) + (.9 \times 0 \text{ lives lost}) = 10{,}000{,}000 \text{ lives lost}$$

The expected utility of Option 2 (i.e., pursuing GOFR) would be:

$$(.05 \times 100{,}000{,}000 \text{ lives lost}) + (.05 \times 40{,}000{,}000 \text{ lives lost}) + (.006 \times 100{,}000{,}000 \text{ lives lost}) + (.004 \times 40{,}000{,}000 \text{ lives lost}) + (.0006 \times 2{,}500{,}000{,}000 \text{ lives lost}) + (.0004 \times 1{,}000{,}000{,}000 \text{ lives lost}) + (.889 \times 0 \text{ lives lost}) = 9{,}660{,}000 \text{ lives lost}$$

According to the expected utility maximization approach to decision-making, we should thus choose Option 2—i.e., proceed with GOFR—because this would lead to a smaller number of expected lives lost.

There are some kinds of cases where an expected utility approach to decision-making might obviously be rational and prudent. Suppose one was offered the following gamble: A fair die is tossed and you receive $7 if it lands on number 6, and you pay $1 if it lands on any other number. The expected utility of not taking this gamble would be $0—i.e., you would not gain or lose any money.[10] The expected utility of taking this gamble would be:

$$(5/6 \times -\$1) + (1/6 \times \$7) = \$0.33$$

According to the expected utility approach to decision-making, one should take the gamble. Assuming that one is not morally opposed to gambling, and that one could play the game as often as one likes, furthermore, it would presumably be rational to do so—because one could expect to win an average of $0.33 per roll of the die.

According to the expected utility maximization approach to decision-making, however, one should take a gamble like this even if it were only offered once—because the expected utility of playing would still be greater than the expected utility of not playing. If one could only play a game like this once, however, then it is highly likely (i.e., there is a 5 in 6 chance) that one would end up losing—so it is not so obvious that it would be irrational or imprudent to refrain from playing. Assuming one can afford to lose $1, on the other hand, it would likewise not obviously be irrational to take one shot at a game like this.

Another, related kind of challenge to the expected utility maximization approach to decision-making (and one that might be especially relevant to GOFR) is revealed by imagining a similar kind of gamble with higher stakes: A fair die is tossed and you pay $100,000 if it lands on number 6, and you win $20,001 if it lands on any other number. The expected utility of taking this gamble would be:

$$(1/6 \times -\$100{,}000) + (5/6 \times \$20{,}001) = \$0.83$$

Despite the positive expected utility of such a gamble, taking it would be considered (highly) irrational by almost everyone (or at least those without millions of dollars to gamble with). For many people, such a gamble would ultimately involve betting one's house, with a fairly high (i.e., 1 in 6) chance of losing it. This objection to the expected utility maximization approach to decision-making is that it

[10] Here, and in what follows, it is assumed that money (gained or lost) can be considered a proxy for utility (gained or lost)—just as number of lives saved or lost might be considered a reasonable (simplifying) proxy for utility in the case of GOFR.

might sometimes be rational/prudent to sacrifice expected utility in order to avoid options with especially costly possible outcomes. The underlying suggestion is that the expected utility maximization approach to decision-making is not sufficiently risk averse. The aim to avoid options with especially costly possible outcomes (even when the option in question maximizes expected utility) gives rise to doubt that GOFR should actually be pursued in the hypothetical example above—the idea being that it would be too risky to pursue a course of action that has a nontrivial possibility of killing 2,500,000,000 people even if expected utility would be maximized by such a course of action.[11]

In any case, it would presumably be impractical to employ the expected utility maximization approach to decision-making in the context of GOFR policy-making—because such an approach requires (1) identification of all the possible outcomes of options, (2) estimation of the likelihood of such outcomes, and (3) estimation of the utility (or value) of each outcome. For reasons discussed above, this would be unrealistic in the case of GOFR (see also Douglas 2013; Resnik 2014). It is impossible to predict, with any confidence, the likelihood of malevolent use (Posner 2004), for example, and there are innumerable scenarios that could result from such use.

Maximin

Another approach to decision-making involves the idea that we should identify the worst possible outcome that might arise from each option under consideration and then choose the option with the best worst possible outcome—i.e., we should choose the option for which the worst outcome is least bad, or we should aim to maximize the utility of the possible outcome with the minimum utility. It is commonly thought that such an approach, referred to as the maximin risk-taking strategy, would be especially appropriate in circumstances where the probability of outcomes that might arise from various options is unknown, but a risk-taking strategy like this could also be considered an alternative to the expected utility maximization approach to decision-making even in cases where the probabilities of option outcomes are estimable. In the latter kind of case, for example, the maximin strategy would call for a decision to refrain from GOFR in the hypothetical H5N1 example considered above, because the worst possible outcome of GOFR (2,500,000,000 lives lost) is worse than the worst possible outcome of refraining from GOFR (100,000,000 lives lost). The maximin strategy also captures the intuition that it would be irrational (for those who are not millionaires anyway) to take the high stakes die gamble.

[11] This kind of objection to the expected utility maximization approach could arguably be addressed by accounting for risk-aversion—or the value of security—in the utility metric (i.e., rather than using number of lives lost or saved as a proxy for utility). Practical difficulties of the expected utility maximization approach (in the context of GOFR) discussed in what follows would nonetheless remain (and perhaps be exacerbated by more complicated utility metrics). Points (below) regarding the importance of democracy to value identification/weighting are likewise arguably applicable to expected utility maximization approaches employing more complicated utility metrics. For discussion of expected utility maximization approaches that aim to capture a plurality of potentially conflicting values, see Paul Weirich (2012).

While the maximin strategy addresses the concern that the expected utility maximization approach to decision-making is insufficiently risk averse, the maximin strategy arguably goes too far in the opposite direction (Hansson 2003). The hypothetical example regarding H5N1 considered above, for example, was designed to suggest that pursuing (at least certain kinds of) GOFR should be considered the option with the worst possible outcome, because (certain kinds of) GOFR might entail the possibility of disaster resulting from pathogens more dangerous than those that otherwise would have arisen. Even when/if this is correct, however, it is not obvious that this should imply that (such cases of) GOFR should never be pursued. Even if we assume that the worst possible outcome of (a certain case of) GOFR is worse than the worst possible outcome of refraining from GOFR, we might nonetheless think that GOFR should be pursued. One could imagine a case of GOFR that:

- is highly likely to have enormous benefits;
- has a worst possible outcome considered to be extremely unlikely (though likelihood of the worst possible outcome may be uncertain and/or exceedingly difficult to estimate with confidence);
- has a worst possible outcome that is not considered to be more likely—and/or is considered to be less likely—than the worst possible outcome of refraining from GOFR (though likelihood of the worst possible outcome of refraining from GOFR is likewise uncertain and/or exceedingly difficult to estimate with confidence);
- has a worst possible outcome that is only just slightly worse than the worst possible outcome of refraining from GOFR.

Though a maximin approach would call for refraining from GOFR in such a case, it is by no means clear that this would be appropriate. A problem with the maximin approach is that it requires maximization of the utility of the worst possible outcome regardless of (1) the cost in terms of forgone benefits, (2) the likelihood (uncertain or otherwise) of the worst possible outcomes of alternative actions, and (3) the extent to which the worst outcome of the option with the best worst outcome is actually better than the worst outcomes of other options.

Maximax

The maximax approach is the polar opposite of maximin. It holds that we should choose the option with the best possible outcome—i.e., we should choose the option for which the best possible outcome embodies the greatest amount of utility, or we should aim to maximize the utility of the possible outcome with maximum utility. Though less widely discussed than the approaches presented above, there are cases where such a decision-making strategy might be considered preferable to either maximin or the expected utility maximization approach to decision-making. One might imagine a case of GOFR that:

- has an expected utility that is slightly less than the expected utility of refraining from GOFR;

- has a worst possible outcome that is slightly worse (and not significantly more likely) than the worst possible outcome of refraining from GOFR;
- has a possible outcome that is much better than any possible outcome of refraining from GOFR (e.g., it is highly unlikely, but possible, that the GOFR in question will lead to a broad spectrum influenza vaccine that prevents enormous numbers of deaths for years to come).

Proceeding with GOFR in a case like this—i.e., following a maximax strategy—might not obviously be inappropriate. Maximax is an ambitious risk-taking strategy that embodies the idea "nothing ventured, nothing gained" (Sunstein 2005). It is arguably the strategy behind at least some blue-sky research—and the (not obviously irrational) strategy employed by those who play lotteries—which usually involve negative expected utility and the worst bad outcome (i.e., loss of a dollar or two) but provide the chance of winning a not otherwise attainable fortune. On the other hand, it is also easy to imagine cases where such an approach would obviously be irrational/imprudent.[12]

Pluralism

Maximum expected utility, maximin, and maximax, might each be legitimate goals. Other things being equal, that is, decision-making should arguably favor the option with maximum expected utility. Other things being equal, decision-making should arguably favor the option with the best worst outcome (maximin). And, other things being equal, decision-making should arguably favor the option with the best possible outcome (maximax).

There may be cases where the very same option promotes all three of these things (maximum expected utility, maximin, and maximax) at the very same time—and in cases like that (which could turn out to include cases of GOFR) it might be quite obvious what should be done. In other cases there might be conflict between these three arguably legitimate goals of decision-making. Such cases raise difficult questions about the weightings that should be attributed to such goals and/or how to strike a balance, or make trade-offs, between them. The hypothetical examples discussed above suggest that the weightings attributable to such goals may be context dependent—e.g., maximin might be especially weighty in high risk situations, maximax might be especially weighty in low risk situations, and expected utility maximization might be especially weighty in cases were multiple attempts (at the gamble in question) are possible and/or in low stake situations (i.e., where the worst possible outcome is not so bad). Different risk-taking strategies (employed by different people), in any case, might attach different weightings to the goals in question—and there may be reasonable disagreement about what, if any, is the correct risk-taking strategy. In a democracy, the risk-taking strategy employed by policy-making should arguably reflect the risk-taking strategies of the people.

[12] Imagine, for example, that ordinary lottery tickets (with ordinary odds and payouts) cost thousands of dollars.

Precautionary Approach

The "precautionary principle", or versions thereof, has often been appealed to in contexts of uncertainty and catastrophic risk, and debates about environmental dangers in particular. A relatively weak, and not especially controversial, version of the precautionary principle is adopted by the *Rio Declaration on Environment and Development*:

> Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation (United Nations Conference on Environment and Development 1992).

This version of the precautionary principle is partly a claim about burden of proof, the idea being that we need not have certainty that a given course of events will lead to great harm in order to justify taking preventative action against the potential dangers in question. In the context of GOFR, such a version of the precautionary principle would entail that uncertainty about dangers regarding biosafety and/or malevolent use would not provide reason (e.g., in decision- and policy-making) to ignore the potential dangers in question. This version of the precautionary principle is considered relatively weak, however, because it does not clearly imply an especially high degree of risk aversion, and it would not (necessarily) rule out potentially risky GOFR.

Stronger versions of the principle, however, are (akin to the maximin approach) more clearly risk averse. The strongest version of the precautionary principle would hold that we should not take actions that pose serious dangers (where likelihood of the dangers in question is uncertain). Cass Sunstein (2005) argues that such a strong version of the principle would be incoherent, because serious dangers will be possible outcomes of any course of action. In the context of the environment, it might be thought that this strongest version of the precautionary principle speaks against developing and/or releasing genetically modified organisms (GMOs), because their development/release might pose serious (though, admittedly, uncertain) dangers. Sunstein (2005), however, has noted that the failure to develop and/or release GMOs might likewise pose serious (though, admittedly, uncertain) dangers, because it might turn out that GMOs enable avoidance of major famines that would otherwise occur. The strongest version of the precautionary principle would thus apparently (also) entail that we should not refrain from GMO development/release. In the context of GOFR one might argue that, according to the strongest version of the precautionary principle, we should not pursue GOFR because GOFR may lead to serious dangers involving laboratory accidents or malevolent use. By the same token, however, one might argue that we should pursue GOFR because GOFR might enable control of pandemics that would otherwise be disastrous. The strongest version of the precautionary principle thus appears to give conflicting advice regarding both GMOs and GOFR, and thus no guidance at all. Sunstein argues that people's appeal to the strongest version of the precautionary principle can be explained by the fact that they are more attuned to some kinds of dangers than others, due to cognitive bias.[13]

More moderate versions of the precautionary principle hold that we should avoid actions (or courses of action) when the dangers they pose are not merely serious, but exceed severity thresholds. Sunstein (2005), for example, appeals to an Anti-Catastrophe version of the precautionary principle according to which we should avoid courses of action that pose catastrophic dangers in particular. Because catastrophe might possibly result from any course of action he furthermore argues that the likelihood of catastrophe, though uncertain, would need to exceed a likelihood threshold—i.e., the catastrophic danger in question, though uncertain, would need to be sufficiently likely (as opposed to a theoretical or minutely remote possibility)—in order for the Anti-Catastrophe version of the principle to take effect. Though moderate versions of the precautionary principle like this might be more plausible than strong versions of the precautionary principle, questions remain regarding how likely a catastrophic risk would need to be in order for such a principle to take effect (i.e., where, exactly, should the likelihood threshold be set?). It also raises questions about the magnitude of harm that should divide catastrophic (or, in other moderate versions of the precautionary principle, sufficiently serious) dangers from others. Different risk-taking strategies, embodying different levels of risk aversion, will set such thresholds in different places.

Even moderate versions of the precautionary principle might arguably, depending on where thresholds are set, be implausibly risk averse—i.e., by entailing that there are certain courses of action that we should never pursue regardless of their expected benefits. Moderate versions of the precautionary principle, finally, like stronger versions, might sometimes provide conflicting guidance (and thus be incoherent/paradoxical) (Clarke 2013). If both (a certain case of) GOFR and the failure to pursue (a certain case of) GOFR pose nontrivial though uncertain dangers of catastrophe beyond thresholds for likelihood and severity of harm, then even moderate versions of the precautionary principle, such as that advocated by Sunstein, would entail both that we pursue and that we refrain from pursuing the GOFR in question.

Frida Kuhlau and colleagues (2011) have developed/proposed a specific version of the precautionary principle, for dual-use life science research in particular, that holds:

> When and where serious and credible concern exists that legitimately intended biological material, technology or knowledge in the life sciences pose threats of harm to human health and security, the scientific community is obliged to develop, implement and adhere to precautious measures to meet the concern (p. 8).

While David Resnik (2013) likewise appeals to the precautionary principle in the context of dual-use research, on his view

> the basic idea of the precautionary principle is that we should take *reasonable* measures to avoid, minimize, or mitigate harms that are plausible and serious (p. 28, my emphasis).

[13] E.g., the "availability heuristic"—i.e., in light of past experience, some dangers more readily come to mind than others, so these become targets of what are actually disproportionate precautionary attitudes.

In responses to Kuhlau and colleagues, that might also apply to Resnik, Steve Clarke highlights the importance of clarity regarding the role that the precautionary principle is meant to play in decision- and policy-making. Precautionary principles described by Kuhlau and colleagues and Resnik might sound reasonable if they are meant to supplement rather than replace cost-benefit analysis (CBA)[14]; but why, asks Clarke (2013, pp. 231–232), should we think that CBA requires such supplementation to begin with? Would a cost-benefit approach to dual-use life science research (and/or GOFR) deny that such research poses plausible serious risks warranting serious remedies and/or exclude such risks from consideration; and is appeal to the precautionary principle thus necessary to address an actual gap in CBA?

If Kuhlau and colleagues intend to suggest a stronger precautionary principle that is meant to replace (rather than merely supplement) CBA, on the other hand, then Clarke argues that their principle would (like other strong versions of the precautionary principle discussed above) be (1) implausibly insensitive to forgone benefits associated with precautionary action and (2) likely to give conflicting guidance.

Rights-Based Approach

Beyond utility risks and benefits, a crucial point of Sven Ove Hansson (2003) is that equity and rights are essential to risk-related decision- and policy-making. Just as it would (usually) be rights-violating and thus unethical for one person (or group) to harm another, it might at first glance be thought that it would be unethical for one person (or group) to impose risk of harm on others (in the absence of explicit consent). Because ordinary action—e.g., driving one's car down the street (without explicit consent of residents)—involves imposing risks on others, however, it cannot be the case that every instance of risk imposition on others (in the absence of explicit consent) constitutes unethical action (Hansson 2003). We mutually benefit by allowing one another to impose (certain) risks on each other (in the absence of explicit consent); and if imposition of risks on others were ruled out, by ethics or policy, then human life would come to a standstill. This raises the question of what should be considered ethically acceptable risk imposition. Hansson argues that

[14] Cost-benefit analysis (CBA) (presumably, for Clarke) involves a decision procedure along the lines of expected utility maximization. CBA often involves conception of utilities in monetary terms in particular (Sunstein 2005), but it is not obvious that this is what Clarke has in mind. Clarke characterizes CBA as follows: "CBA involves attempting to determine the probability of benefits occurring, and the probability of costs being incurred, as well as determining the relative sizes of the benefits and costs of a particular course of action and balancing these. This calculation is compared with the relative balance of costs and benefits for alternative courses of action from which the option with the best overall balance, adjusting for probability of these occurring, is selected" (Clarke 2013, p. 224). That Resnik's use of the precautionary principle involves conjunction with (rather than replacement of) cost-benefit thinking is revealed by his use of "reasonable" and his actual analysis. Resnik's idea obviously is not that we must do whatever it takes to avoid dangers posed by dual-use research at any cost; his analysis reflects the idea that costs and benefits of precautionary action need to be considered (and the idea that balance between costs and benefits determine what is "reasonable"). Clarke might argue that Resnik's precautionary approach sounds like a re-description of cost-benefit thinking rather than a replacement thereof.

> [while] everyone has a prima facie moral right not to be exposed to risk … this right can be overridden if [and only if] the risk-exposure is part of an equitable system for risk-taking that works to the advantage of the individual risk-exposed person (p. 291).

This kind of approach is preferable to the underlying utilitarian thinking behind expected utility maximization, according to Hansson, because the latter kind of approach (in addition to other objections raised above) is insensitive to human rights and distributive/egalitarian concerns.[15] Risks associated with GOFR, according to Hansson's approach, might be considered acceptable if the scientific and technological enterprise (if that is meant to be the "system of risk-taking" in question) equitably benefited all of those exposed to the risks involved. Given global political economics, some might doubt that this is the case—because some people exposed to the risks involved benefit more from scientific and technological advance than others. If Hansson's principle is taken to be absolute, then risks associated with the scientific and technological enterprise would be considered unacceptable if such doubts about equity are justified. If a degree of inequity is inevitable (in light of global political economics) but the scientific and technological enterprise nonetheless perhaps enormously benefits the vast majority of (though not all) people exposed to the risks involved, then one might think that the risk imposition in question is actually justified. If Hansson's principle is absolute, then it would apparently always prioritize equity over utility, but it is plausible that small compromises regarding equity are at least sometimes outweighed by large utility gains (Selgelid 2009b). A more moderate (and less binary) principle than that defended by Hansson (but which nonetheless remains sensitive to rights and distributive/egalitarian concerns) might run as follows: while everyone has a prima facie moral right not to be exposed to risk, over-riding of this right is ethically acceptable *to the degree that* risk-exposure is part of an equitable system for risk-taking that works to the advantage of risk-exposed persons. Such a principle would be more tolerant of risks associated with the scientific and technological enterprise (and thus at least some cases of GOFR).

Deontological Ethics and Double Effect

Deontological approaches to ethics hold that some actions—e.g., intentionally killing an innocent person—would never be morally permissible regardless of the consequences of the action in question. Given the relevance of intentions to the moral permissibility of actions according to (many) deontological ethical frameworks, the "doctrine of double effect" (DDE) is meant to provide "a guide to

[15] The point being that the expected utility approach to decision- and policy-making ultimately aims at aggregate utility maximization without paying sufficient attention to (1) whether expected utility maximization entails rights violations or (2) whether or not utility is fairly distributed. Whether or not this is a fair criticism of expected utility maximization perhaps partly depends on how broadly "utility" is conceived—i.e., because disvalue of rights violations and/or inequality could arguably be factored into utility calculations in various ways. Hansson's criticism of expected utility maximization might be fair, however, if utility is more narrowly conceived in terms of well being (or, as in examples offered above, the number of lives saved or lost).

decision making in ethically difficult cases where an action or course of action with an intended good effect can also produce a foreseen bad effect" (Uniacke 2013, p. 153). DDE holds that it may be morally permissible to pursue an action with a foreseen bad effect so long as the action in question is not itself morally problematic, the bad effect is not itself intended (it is merely foreseen), an intended good effect is directly produced by the action in question (and not directly produced by the bad effect), and this intended good effect outweighs the foreseen bad effect (the proportionality condition) (Uniacke 2013, p. 155). Suzanne Uniacke illustrates the application of DDE to a case where a driver must swerve a car into the path of an innocent pedestrian in order to avoid crashing into a crowd. According to DDE, this might be ethically permissible because the killing of the pedestrian is merely foreseen rather than intended, the saving of the crowd is brought about by the swerving of the car (rather than being caused by the death of the pedestrian), and the many lives saved outweigh the one life lost.

As demonstrated by Uniacke, there are obvious similarities between scenarios where DDE is commonly invoked and the dual-use problematic.[16] In the context of dual-use research, responsible scientists (and/or their funders) intend to conduct (or enable) work that will be benefit humanity (i.e., produce good effects); but they may foresee, though they do no intend, that malevolent use of the research may lead to grave harm (i.e., produce bad effects). Should DDE thus apply to dual use dilemmas? This partly depends on whether DDE is a plausible principle—which has been the subject of much ethical controversy.[17] In any case, Uniacke points out numerous differences between scenarios where DDE is commonly thought to apply and dual use dilemmas:

- In prototypical DDE scenarios the foreseen bad effect is (usually) expected with certainty or high probability, but in the dual use context bad effects are merely a foreseen possibility (and/or presumably often considered to be low probability).
- In prototypical DDE scenarios the foreseen bad effect is (usually) directly produced by the moral agent in question, but in the dual use context the foreseen possible bad effect would result from the malevolent action of others.[18]

Despite these differences, Uniacke argues that DDE framing of dual use dilemmas aptly highlights the *moral responsibility* that scientists (and/or their funders) would have for harms they both *foresee* and *enable*.[19] An implication,

[16] Though Uniacke specifically considers application of DDE to scientists engaged in dual-use research where malevolent use of research findings is a foreseen possibility, much (but not all) of her analysis arguably also applies to (1) funders of research and (2) GOFR biosafety concerns. I add reference to funders in what follows.

[17] Among other objections to DDE, critics commonly highlight difficulties distinguishing intended from merely foreseen consequences (which application of DDE requires).

[18] Though Uniacke specifically focuses on concerns about malevolent use, possible laboratory accident (a foreseeable bad effect) in the case of GOFR could be directly produced either by the moral agent/scientist in question or others (e.g., in the case of proliferation).

[19] Such moral responsibility would not necessarily entail moral blameworthiness. Moral agents in DDE scenarios are morally responsible for foreseen harms that they bring about—but they are arguably not morally blameworthy (if DDE conditions are met).

according to Uniacke, is that scientists engaged in such work (and presumably those funding it) have a moral obligation to ensure that risks associated with dual-use research they conduct (or fund) are minimized. It might also be argued that a version[20] of the proportionality condition of DDE—i.e., that intended/expected benefits should outweigh foreseen harms (or risks)—should also apply to dual-use research.

Principlism

Research Ethics

A number of popular approaches to bioethics appeal to principle-based frameworks. In the context of biomedical research involving human subjects, for example, the Belmont Report (DHHS 1979) argues that judgments about the ethics of research should be guided by the following overarching ethical principles:

- Respect for persons, which requires acknowledgement/respect of individual autonomy and protection of those with diminished autonomy. Application of this principle entails obligations regarding informed consent—i.e., "[human] subjects [of research], to the degree that they are capable, [should] be given the opportunity to choose what shall or shall not happen to them."
- Beneficence, which requires that researchers "(1) do no harm and (2) maximize possible benefits and minimize possible harms." Application of this principle entails "systematic assessment of risks and benefits"; that research involving human subjects "be justified on the basis of a favorable risk/benefit assessment" and/or "that risks to subjects be outweighed by the sum of both the anticipated benefit to the subject, if any, and the anticipated benefit to society in the form of knowledge to be gained from the research."[21]
- Justice, which requires fair sharing of the benefits and burdens of research involving human subjects. Application of this principle requires "fair procedures and outcomes in the selection of research subjects," i.e., those exposed to the risks of research.

Though explicitly designed to provide guidance regarding the ethical conduct of research involving human subjects in particular, it has been argued (Evans et al. 2015; Lipsitch and Galvani 2014) that the Belmont Report's (and also the Nuremburg Code's) beneficence requirements—e.g., that benefits outweigh risks, and that risks should be minimized—should also apply to GOFR. While this might be plausible, it might not be so obvious that Belmont's informed consent requirement could or should straightforwardly apply to GOFR, because it would be impossible to seek/gain individual consent from all "capable" persons exposed to possible risks of GOFR. In the context of GOFR, it might be argued that Respect for Persons alternatively requires community consent and/or democratic processes.

[20] Taking probabilities into account.

[21] Similar claims about the need for benefits to outweigh risk are embodied by other human research ethics frameworks (such as The Nuremburg Code) and US federal regulations.

Biomedical Ethics

A similar ethical framework developed and popularized by Tom Beauchamp and James Childress (2001) for biomedical ethics more generally appeals to a similar set of principles:

- Autonomy: individual autonomy should be respected/promoted.
- Non-maleficence: do not harm others.
- Beneficence: benefit others by protecting/promoting their well being.
- Justice: benefits and burdens should be shared fairly.

The Beauchamp and Childress framework largely mirrors that of the Belmont Report, but Beauchamp and Childress separate what is captured by the Belmont Report's Beneficence principle into two separate principles (Non-maleficence and Beneficence). Beauchamp and Childress acknowledge that there may sometimes be conflict between their principles, and that a balance should, in such cases, be struck between them. If GOFR is expected to be especially beneficial (let's assume, for example, the overall benefits for humanity outweigh the risks) but inevitably entails compromised autonomy (because it entails imposition of risk on individuals without their explicit consent) then the beneficence principle would conflict with the autonomy principle. The above discussion of Hansson likewise illustrates how beneficence might conceivably conflict with justice in the context of GOFR. The possibility of conflict between principles raises difficult questions about what would be a principled/legitimate way to strike a balance, or make trade-offs, between them (or the values they embody) in such cases.

Public Health Ethics

Recently developed frameworks for public health ethics are explicitly designed to address possible conflicts between liberty and utility that arise in cases where coercive (i.e., liberty-infringing) measures such as isolation and/or quarantine are necessary to protect/promote public health.

Among other things, public health ethics frameworks (Gostin 2006; Kass 2001; Selgelid 2009a; Upshur 2002) have posited that (1) liberty restriction in the name of public health protection should be based on evidence that the public health measure in question would in fact provide an effective means of public health protection, (2) the least restrictive (i.e., least liberty-infringing) alternative should be employed to achieve the public health goal in question, (3) extreme liberty-infringing methods such as isolation and quarantine should not be employed unless the consequences would otherwise be severe, (4) liberty-infringing interventions should be used in an equitable—i.e., non-discriminatory—manner and/or the bar for imposing such measures should be highest (with regard to the evidence required or the utility threatened) when those being considered for confinement are members of the worst off groups of society, (5) liberty-infringement should be minimally burdensome (e.g., so that those confined receive basic necessities and are made as comfortable as possible), (6) those whose liberty is violated should be compensated in return (7)

implementation of liberty restrictions should involve due (legal) process, and those confined should have a right to appeal, and (8) relevant policy-making should (insofar as possible) be democratic and transparent.

Because imposition of risk on individuals could be conceived as a form of liberty-infringement, such principles (if legitimate) may have relatively straightforward application to the context of GOFR (aimed at public health protection/promotion). In any case, imposing risks on individuals without their (explicit) individual consent (in the case of GOFR aimed at public health protection/promotion) might be ethically problematic in a way that is similar[22] to what is problematic about coercive public health measures. If this is correct, then it would not be surprising if analogous principles applied to the two kinds of cases.

In contrast with Beauchamp and Childress' principlist framework, which is designed to highlight prima facie principles/values that should be satisfied/promoted when possible (rather than constituting necessary conditions), the public health principles outlined above are commonly framed as necessary conditions—each of which, it is argued, must be satisfied for liberty restriction aimed at public health promotion/protection to be ethically acceptable. Application of this kind of framework is not entirely straightforward, because it may often not be obvious whether any given principle is satisfied. With regard to (1), for example, how much and/or what kind of evidence would/should be needed?

Towards an Ethical and Decision-Making Framework for GOFR (Funding) Policy-Making

In light of the preceding discussion of points raised in the GOFR ethics literature, limitations of RBA, and challenges to existing ethical and decision-making frameworks, the following framework might be considered appropriate for decision- and policy-making regarding the funding and conduct of GOFR. This framework is based on the idea that there is likely no (clearly correct) exact formula or algorithm that will solve hard questions about GOFR—and that judgments will inevitably need to be made. It thus highlights ethical desiderata that such judgments should be based upon, i.e., dimensions upon which policy makers (or decisions) could fare ethically better or worse. Because judgments will depend on numerous matters regarding which there is likely to be reasonable disagreement (i.e., matters that cannot be resolved by science and/or the discipline of ethics—e.g., questions about what is intrinsically valuable, the weightings that should be attributed to potentially conflicting values, appropriate levels of risk aversion, and/or appropriate risk-taking strategy), this framework suggests, among other things, that decision- and policy-making regarding the funding and conduct of GOFR should be as democratic as possible. Many of the hard ethical questions raised by GOFR, that is, should be resolved in a way that reflects the values and risk-taking strategies etc. of the people.

[22] i.e., doing potentially damaging things to people, and/or perhaps infringing upon their rights, in the aim to protect/protect public health.

Because the US Department of Health and Human Services (DHHS) has determined that it will only fund GOFR where the expectation is that the study in question will be published (DHHS 2013), it should be noted at the outset that determination that any given study should not be published would entail that the study in question should not be funded by DHHS. Reaching such a determination, however, need not imply judgment that such a study should not take place at all, because studies not funded by DHHS might be funded privately and/or funded by other US government agencies to be conducted in a classified manner. It should also be noted that the decision not to fund any given study (even, ironically, in cases where such a decision is largely or partly based on concerns about publication dangers) is arguably less weighty than the decision to censor a study would be. Censorship involves direct interference with the scientific enterprise, academic freedom, and/or freedom of speech. While this does not necessarily mean that censorship would always be wrong it does mean that the grounds for censorship would need to be stronger than grounds for refraining from funding (a case of) GOFR—because refraining from funding (a case of) GOFR would not involve direct governmental interference with the scientific enterprise, academic freedom, or freedom of speech. The decision not to fund (a case of) GOFR might sometimes reflect the conclusion that (in light of an all-things-considered assessment of benefits and risks involved) there might be better uses of taxpayers' money. Whether or not GOFR is involved, one should expect policy makers to consider possible risks/harms as well as benefits when making decisions about what research to fund (World Health Organization 2010). These preliminary remarks are by no means intended to downplay the potential value/importance or fundability of GOFR in general. As with non-GOFR studies, some (proposed) GOFR studies may be more socially valuable, and thus more worthy of funding, than others.

Research Imperative

In cases where it is determined that GOFR (or publication thereof) may pose extraordinary risks to the public (or groups therein), the GOFR in question would be morally problematic. The ethical acceptability of GOFR (and publication thereof) thus partly depends on the extent to which there is an important reason to conduct (and publish) the GOFR in question. This principle appears to entail that, to be ethically acceptable, extraordinarily risky GOFR must address an important public health question. Conceived in a binary way (as in the previous sentence), however, a principle like this would be difficult to implement, because it raises arguably intractable questions about exactly how risky a study would need to be in order to be considered extraordinarily risky and exactly how important the research question would need to be in order for the research to satisfy the criterion in question.

Conceived as a scalar moral desideratum (rather than as a necessary condition/criterion that is either satisfied or not satisfied) the point of this principle is that, in cases where the research poses serious risks, its evaluation should *partly* be based on the importance of the research question it aims to address. Some research questions are obviously more important than others. The more important any given target research question, the more ethically acceptable it would be to fund/conduct/publish

a study posing a given magnitude of risk (other things being equal). The less important any given research question would be, the less ethically acceptable it would be to fund/conduct/publish a study posing the same magnitude of risk (other things being equal). Generally speaking, furthermore, the riskier the research would be, the more important the research question would need to be in order for the research to be justified (other things being equal).

Proportionality

The ethical acceptability of extraordinarily risky GOFR partly depends on the extent to which there is reasonable expectation that the research in question will (1) yield answers to the target public health question, and (2) ultimately result in public health benefits that outweigh risks involved. In any given case (depending on RBA findings) we might be more or less confident that the GOFR in question will actually satisfy these two conditions. Conceived as a scalar moral desideratum (rather than as a necessary condition/criterion that is either satisfied or not satisfied) the point of this principle is that, in cases where the research poses serious risks, its evaluation should *partly* be based on the level of confidence that (1) and (2) are satisfied. The greater confidence that (1) and (2) are satisfied, the greater the ethical acceptability of funding/conducting/publishing a study posing a given magnitude of risk—and vice versa. Other things being equal, furthermore, the greater the expected benefits of any given case of GOFR posing a given magnitude of risk, the more ethically acceptable it would be to fund/conduct/publish the study in question.

Minimization of Risks

The idea that research risks should be minimized is a central tenet of human subjects research ethics. A call for risk minimization has likewise been widely appealed to in debates surrounding GOFR; and numerous ways in which risks related to GOFR might be minimized have been identified in the literature.

This kind of principle parallels the "least restrictive alternative" principle commonly appealed to in public health ethics. The latter holds that it would be unethical to employ more force/coercion than is necessary to achieve the public health goal in question—i.e., among alternative public measures that are otherwise ethically acceptable and equally effective, the measure involving the least force/coercion should be chosen. The least restrictive alternative principle in public health ethics, however, does not (necessarily) imply that a less restrictive measure should be preferred to a more restrictive measure if the former would entail compromised efficacy towards achieving the public health goal at issue.

In the context of GOFR, it is similarly plausible that (other things being equal) risky GOFR should not be pursued unless there is reason to believe that less risky kinds of research are unlikely or unable to *equally well* yield answers to the target public health question and thereby ultimately achieve public health benefits.[23] As in

[23] It is likewise arguable, as suggested by Marc Lipsitch and colleagues, that risks might sometimes be minimized via pursuit of public health activities other than (GOFR) research—e.g., surveillance—that are

the discussion of proportionality, in any given case we might have more or less confidence that a GOFR study is not more risky than other equally beneficial possible research alternatives,[24] so the ethical acceptability of risky GOFR will be a function of the extent to which there is good reason for such confidence.

A further implication of the minimization of risk principle is that *when pursuing GOFR* we should minimize risks (at least insofar as possible without compromising expected benefits of the GOFR study in question). This raises the question of whether risks must be maximally minimized regardless of the (e.g., economic) costs and/or extent of risk reduction achieved—and/or what would be a "reasonable" cost to endure for marginal risk reduction. Again, if stated in binary terms, it is hard to imagine what a precise (plausible) minimization of risk principle should look like.

Conceived as a scalar moral desideratum (rather than as a necessary condition/criterion that is either satisfied or not satisfied) we might thus state this principle as follows: other things being equal, the ethical acceptability of (a given case of) GOFR is a function of the degree to which (1) there is confidence that no less risky forms of research would be equally beneficial (regarding the public health question/problem at issue) and (2) reasonable steps have been made to minimize risks of conducting the GOFR in question. This principle does not (necessarily) imply that a less risky study should be preferred to a more risky study if the former would be less beneficial.

Manageability of Risks

Whether or not any given study should be funded/conducted/published partly depends on existing global "web of prevention" control measures in place rather than depending entirely on essential features of the GOFR study itself. Manageability of GOFR risks, like other relevant features considered above, is a matter of degree rather than either-or. Other things being equal, the more manageable the risks of (any given case of) GOFR (which partly depends on the strength of the background web of prevention in place), the more ethically acceptable the (case of) GOFR would be. Conversely, the more important/beneficial (any given case of) GOFR is expected to be, the more we should be willing to accept potentially unmanageable risks. It is also worth noting that severity of potentially unmanageable risks is also ethically relevant—because some potentially unmanageable risks might be less severe than others (and some potentially unmanageable risks might not be very severe at all).[25]

Here and in principles above (and below), a purpose of highlighting scalar dimensions of ethically relevant aspects of GOFR (i.e., highlighting that ethically relevant aspects of GOFR come in degrees rather than being either-or) is to reveal

Footnote 23 continued
equally (or more) beneficial than the GOFR under consideration. This point is implicitly addressed by the Research Imperative principle, because the importance of a research question is largely a function of the extent to which answering it is crucial to achievement of public health goals.

[24] A similar point in the context of "least restrictive alternative" is made by Timothy Allen (unpublished).

[25] Though arguably unmanageable, the weeds in my garden are tolerable.

that: (1) appeal to either-or/binary criteria might not be sufficiently clear or action guiding (insistence that "risks of GOFR must be manageable or reasonably manageable", for example, is arguably prohibitively vague); and (2) strict insistence on certain criteria might rule out too much. With regard to (2) we might imagine cases of especially important/beneficial GOFR—i.e., addressing crucial (and potentially otherwise unmanageable) risks—that it might be appropriate to pursue even if the GOFR in question poses nontrivial risks of unmanageability with nontrivial severity (though less unmanageability and less severity than the risks that the GOFR aims to address). (Some might think this, for example, about the controversial ferret H5N1 influenza studies.) As noted above, the acceptability of unmanageability of (any given case of) GOFR depends on the costs (in terms of forgone benefits) of refraining from (the case of) GOFR (in question).

Justice

Justice requires fair sharing of research benefits and burdens. It would arguably be unjust if (1) GOFR risks fall upon some people (e.g., those living in countries with weak health care systems) more than others, (2) GOFR risks fall upon those who are unlikely to benefit from the research in question, and/or (3) individuals or groups suffer harms from GOFR without being compensated. As argued above in discussion of Hansson, though a perfectly equitable sharing of the risks and benefits of GOFR might be unrealistic given global political economics, it is reasonable to believe that the ethical acceptability of GOFR is a function of equity. Other things being equal, the more that is done to ensure equitable sharing of risks and benefits, the more ethically acceptable GOFR would be. Other things being equal, the less that is done to ensure equitable sharing of risks and benefits, the less ethically acceptable GOFR would be. Among other things, such a principle implies that the ethical acceptability of GOFR is a function of the degree to which (wealthy) countries conducting/funding GOFR (1) mitigate risks for those who are especially vulnerable (both domestically and internationally), (2) ensure wide availability of GOFR research benefits (both domestically and internationally), and (3) compensate those who suffer harm resulting from GOFR (both domestically and internationally).

Good Governance: Democracy

The above discussion reveals numerous ways in which decision- and policy-making regarding GOFR turns on important, difficult questions—about ultimate values, value weightings, and risk-taking strategies, etc.—regarding which there will inevitably be reasonable disagreement. In a democracy, decision- and policy-making regarding GOFR should arguably (as far as possible) reflect the ultimate values, value weightings, and risk-taking strategies of the people (Kitcher 2001).

In addition to expert opinion (which is inevitably necessary), therefore, GOFR policy-making should involve systematic ongoing engagement with key stakeholders and the community at large—via processes of deliberative democracy[26]—in order to gain direct public input to decision-making and learn more about the

ultimate values, value weightings, and risk-taking strategies that the public would like to see (and that thus should be) reflected/implemented by policy.

While individual informed consent to GOFR risks is obviously infeasible, community consent might address the Belmont Report's (and other research ethics codes') requirement of respect for persons—and deliberative democracy might be an ideal method for seeking community consent. Decision- and policy-making should, in any case, be as transparent as possible—because transparency plays a crucial role in democratic processes (Sen 1999).

In addition to being ethically important, democratic decision-making is important because democratic decision-making is necessary to maintain/improve public confidence and trust in both the scientific enterprise and government. Public trust and confidence are values that could be compromised (with adverse consequences) whether or not GOFR results in untoward outcomes. Such values may be compromised if the public is not satisfied that GOFR policy decisions adequately reflect the will (i.e., values, value weightings, risk-taking strategies, etc.) of the people and/or if it appears that GOFR policy entails unjust rights violations and/or is inequitable.

Susan Wolf and her colleagues (2009) and the Institute of Medicine (IOM) Committee on the Independent Review and Assessment of the NIH Recombinant DNA Advisory Committee (RAC) (IOM 2014) document the valuable role RAC has historically played in the promotion of public dialogue concerning ethical and social issues pertaining to gene transfer research involving human subjects. This has been achieved by its public review of especially challenging research protocols. The IOM RAC Report explicitly recommends considering possible establishment of a similar kind of venue for other emerging technologies raising important/difficult social and ethical issues. The IOM RAC Report suggests that such a venue might:

- Provide a public forum for the review and discussion of emerging areas of science
  - Include the capacity for a partnership to consult, inform, and educate institutional review boards (IRBs) and institutional biosafety committees (IBCs).
- Provide a venue to foster scientific and public awareness regarding emerging science in order to address concerns about clinical investigations and future societal implications.
- Integrate the capacity to surveil, aggregate, and analyze adverse events across related trials of emerging technologies.
- Perform an additional level of review of individual protocols that are identified by the NIH director, in consultation with one or more IRBs and IBCs, on the basis of exceptional issues raised (IOM 2014, pp. 6–7).

[26] That GOFR policy making should involve deliberative democracy has also been suggested by David Relman (Duprex et al. 2015).

Though the IOM RAC Report is here explicitly referring to a venue concerned with *clinical research* involving new emerging technologies, analogous roles to many of those described above should arguably be filled by a relevant body in the context of GOFR,[27] and more explicitly broadening the mandate of NSABB to fulfill such roles would be an obvious possibility.[28] Whether or not *public review* of protocols, in particular, would be advisable in the context of GOFR is clearly open to question, because this could itself pose dual use dangers (via dissemination of potentially dangerous information and/or by promoting GOFR proliferation in worrisome kinds of cases).

Evidence

The above discussion reveals that the ethical acceptability of GOFR depends on confidence regarding the (potentially unique) benefits and risks of conducting GOFR (in particular ways), how risks can be minimized,[29] who might be likely to benefit or be harmed by the research in question, and the values and risk-taking strategies etc. of the people, which policy should aim to reflect. Confidence about such matters depends on the current state of knowledge, which can be improved via relevant empirical research. In some cases crucial ethical/policy decisions turn on answers to what are ultimately empirical questions. Answering such questions may thus be both scientifically and ethically important (Selgelid 2009a).

The RBA currently underway is a step in the direction of better-informed GOFR decision- and policy-making. Similar and/or relevant research (RBA and otherwise) concerning GOFR in general—and/or particular kinds of cases of GOFR—should continue in the future and receive relevant funding as necessary. The better informed any decision in favor of (or against) GOFR, the more ethically acceptable the conduct (or omission) of that GOFR would be.

Beyond processes of deliberative democracy, furthermore, carefully designed social research will be important for shedding light on people's (reflectively held, as opposed to cognitively biased) ultimate values, value weightings, levels of risk aversion, and risk-taking strategies etc. that policy should aim to reflect.

Among other things, finally, the evidence principle entails careful ongoing monitoring of GOFR (e.g., with an eye to adverse events and compliance with safety protocols)—and it might sometimes require acquisition of and/or access to potentially classified intelligence information about the abilities, possessions, and intentions of malevolent actors or groups.

[27] Imperiale and Casadevall (2015, p. 5) have similarly suggested the possibility of "[c]reation of a national board to vet issues related to research with dangerous pathogens … [modeled] after the Recombinant DNA Advisory Committee. Such a board should have microbiological, infectious disease, biosafety, and ethical expertise, which, combined with access to national security information, would allow better assessments of biosafety and biosecurity issues." For related proposals, see Selgelid (2007) and Miller and Selgelid (2008).

[28] At least in cases where the roles in question are not already part of NSABB's current mandate.

[29] The need for additional biosafety research (and associated funding) in particular is also suggested by Evans and colleagues (2015). I here additionally have in mind research that helps determine whether GOFR (as opposed to other kinds of less risky research) is needed to answer key scientific questions.

International Outlook and Engagement

Because the risks and benefits of GOFR (can) affect the global community at large, the ethical acceptability of GOFR at least partly depends on the extent to which such research is accepted abroad. Decision- and policy-making regarding GOFR should arguably, insofar as is feasible, involve consultation, negotiation, coordination, and related forms of active engagement with other countries.

In its report *New Directions: The Ethics of Synthetic Biology and Emerging Technologies* The Presidential Commission for the Study of Bioethical Issues, for example, recommends

> International Coordination and Dialogue … Recognizing that international coordination is essential for safety and security, the government should act to ensure ongoing dialogue about emerging technologies such synthetic biology. As part of [a] coordinated approach [the US Government] should continue and expand efforts to collaborate with international governments, the World Health Organization, and other appropriate parties, including international bioethics organizations, to promote ongoing dialogue about emerging technologies such as synthetic biology as the field progresses (Presidential Commission 2010, pp. 10).

This kind of recommendation is directly applicable to GOFR in particular (not least because GOFR will often itself involve synthetic biology).

Conclusion

The ethical- and decision-making framework suggested above is based on the idea that there are numerous ethically relevant dimensions upon which any given case of GOFR can fare better or worse (as opposed to there being necessary conditions that are either satisfied or not satisfied, where all must be satisfied in order for a given case of GOFR to be considered ethically acceptable). Rather than drawing a sharp bright line between GOFR studies that are ethically acceptable and those that are ethically unacceptable, this framework is designed to indicate where any given study would fall on an ethical spectrum, where imaginable cases of GOFR might range from those that are most ethically acceptable (perhaps even ethically praiseworthy or ethically obligatory) (i.e., those that fare best with respect to all 8 dimensions), at one end of the spectrum, to those that are most ethically problematic or unacceptable (i.e., those that fare worst regarding all 8 dimensions, and thus clearly should not be funded/conducted), at the other. The aim should be that any GOFR pursued (and/or funded) should be as far as possible towards the former end of the spectrum.

One reason for resisting an approach based on necessary conditions is that the desiderata highlighted above involve ethically important factors that come in degrees, and it is hard to imagine that there are actually clear thresholds separating adequate from inadequate achievement of any given desideratum. In any given case of GOFR, our epistemic situation regarding achievement of any given desideratum

will likewise be a matter of degree—i.e., there will be greater or lesser confidence regarding achievement level of each desideratum—and it is hard to imagine there being thresholds separating adequate from inadequate confidence. Another reason for resisting a framework based on necessary conditions is the intuition that compromised/suboptimal achievement of some desiderata might sometimes be compensated by high-level achievement of others.

Though the framework suggested here admittedly does not provide an algorithmic guide to action, it is doubtful that any clear algorithmic approach to evaluating GOFR would be justifiable or should be considered realistic or desirable. With regard to desirability, it is noteworthy that an algorithmic approach that merely aimed to separate ethically acceptable from ethically unacceptable cases of GOFR would fail to capture the degree to which any given study is acceptable or not. In cases of GOFR that fall at ends of the ethical spectrum, the framework suggested here (like an algorithmic approach) may give very clear guidance about what should be done. In cases of GOFR that fall in the middle/grey area, difficult judgments will need to be made, and, aside from the aim to achieve a democratic outcome (which should be an especially important desideratum), there might not always be clear right answers regarding whether a given case of GOFR should proceed (or be funded). Like risk-benefit assessment, ethics involves inevitable uncertainty.

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Science

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U.S. Infectious Disease Chief Urges Flu Scientists to 'Engage,' Support H5N1 Research Moratorium

No end in sight for self-imposed ban

31 JUL 2012 • BY ELI KINTISCH, DAVID MALAKOFF

More moratorium. NIAID Chief Anthony Fauci says more discussion is needed before the H5N1 research moratorium can be lifted.

NEW YORK, NEW YORK—A voluntary moratorium on potentially dangerous experiments aimed at understanding highly virulent strains of the H5N1 influenza virus should continue for the time being, National Institute of Allergy and Infectious Diseases (NIAID) Director Anthony Fauci told a meeting of flu scientists

here. But, he added, scientists should redouble their efforts to engage with the larger public to gain support for the vital but risky work.

"The flu scientific community can no longer be the only player in the discussion about this research," Fauci said. "You will unquestionably lose the battle for public support for your research if you ignore this issue." Fauci remarks, delivered at the annual meeting of the NIAID's influenza research centers of excellence, also echoed a call for openness and transparency he made in June in the pages of *Science*, published by AAAS, which also publishes *Science*Insider.

The moratorium, announced by 39 scientists this past January, came amidst controversy over publishing two studies that described how researchers made H5N1 more transmissible between mammals—possibly setting the stage for a flu pandemic. After a lengthy review, the U.S. National Science Advisory Board for Biosecurity (NSABB) ultimately recommended that the U.S. government allow full publication of both studies. One, by a team led by Yoshihiro Kawaoka—who has a joint appointment at the University of Tokyo and the University of Wisconsin, Madison—was published by *Nature*. The other, from a team led by Ron Fouchier of Erasmus MC in Rotterdam, the Netherlands, was published by *Science*.

What to do about the moratorium, however, has been the subject of controversy. It was originally supposed to last just 60 days, but it was later extended indefinitely. In April, Fauci -- who is not a signatory to the moratorium but leads a major flu research funding agency and encouraged scientists to adopt the pause in a bid to calm public fears—told a U.S. Senate committee that the moratorium should continue pending further discussion. In June, he said the research community still had "a lot of homework to do … and some boxes to check" before the moratorium could be lifted, including agreeing on what types of flu research were worth the risks.

In today's remarks, Fauci highlighted one particularly sensitive research area: so-called "gain-of-function" experiments that allow scientists to create and study flu viruses that are more pathogenic than those found in nature. A key argument for doing such experiments, he noted, is that they allow scientists to understand how a virus might evolve in the future.

"There is a real and present danger of the natural evolution of the virus and that is why you do the experiments that might appear to be risky in the eyes of some," he said. "You do the experiments so we can stay ahead of the naturally evolving risk."

Many critics, however, have questioned whether such experiments are really useful, and whether scientists can safely contain potentially dangerous new pathogens. "The world sees it differently," Fauci said, "and they ask the question, … namely: Should these experiments should have been performed and/or published in the first place?"

Scientists, he said, often "answer that the benefit outweighs the risk. … However, it is essential we respect the concern of the public domestically or globally, and not ask them to take the word of the influenza scientist."

Along with concerns about bioterrorists using published papers to guide deliberate efforts to stoke a pandemic, Fauci said he worried about "unregulated" laboratories, perhaps outside of the United States, doing work "sloppily" and leading to an inadvertent pandemic. "Accidental release is what the world is really worried about," he said.

To address such concerns, Fauci counseled more dialogue and patience. Before lifting the moratorium, scientists need to take more time to share information with the public and discuss the tradeoffs inherent in influenza research, he said, perhaps through international workshops and meetings.

Not taking such steps, he said, would be counterproductive. "If we, without having this broader input, say, 'Let's lift the moratorium,' the consequences of that would make it more difficult to get back on the track of doing this research," he said.

Fauci also suggested that there are plenty of potentially important H5N1 experiments that aren't covered by the moratorium. Allowable experiments, for instance, could test the assumption that an influenza virus infecting a small mammal such as a ferret could also infect a monkey. Or, he said, researchers could start more studies to answer questions about how the immune system responds to the virus.

"The game has changed for pandemic flu scientists and the agencies that support them," he said. NIAID, the National Institutes of Health, and the Department of Health and Human Services "cannot recommend or go along with the lifting of the moratorium . . . as long as the questions that I have just mentioned and brought up remained unanswered."

That stance got a predictably mixed review from members of the audience, which included both supporters and opponents of lifting the moratorium. But several scientists publicly thanked the NIAID director for his frank comments. "I think [Fauci] articulated the case for continuing the moratorium today better than the case for continuing the moratorium had been articulated previously, and that's important," virologist Nancy Cox of the Centers for Disease Control in Atlanta told *Science*Insider after Fauci's session. She was among the original moratorium signers. During the session, she also said that "there needs to be a better explanation of what kinds of work on H5N1 can continue under the moratorium and what can't."

Fouchier, for one, told Fauci that it was time for the moratorium to end. "I think we have done what we can do about accidental release," he said. "Accidental release outside of U.S. cannot be addressed by regulation," he noted, adding that some of the signers of the moratorium don't receive U.S. funds. He also pointed out that labs around the world already have dangerous pathogens on hand, including hundreds with samples of the 1957 H2N2 pandemic virus. "If that virus [gets released] it will kill 1 to 2 million people," he said, suggesting that the infectious disease community has shown it can work with dangerous pathogens responsibly.

Fauci's response to Fouchier: Share that argument with the public. What Fouchier said "doesn't get the transparent airing it deserves," Fauci said. "That argument you made has to be made in a forum that people can understand what you are saying."

By the end of the hour-long session, it appeared that researchers were no closer to resolving when—or if—the moratorium should end. "If there is disagreement among the people in this room, how is a decision going to be taken?" asked virologist Adolfo García Sastre of the Mt. Sinai School of Medicine in New York City.

Fauci said that the detailed guidelines it is developing for universities and other institutions to conduct potentially risky dual use research of concern (DURC) will help spell out an answer. As part of his talk, he shared the U.S. government's progress on the DURC guidelines, which have been shaped by a new, yet-to-be-named interagency committee that includes National Institutes of Health officials. The group has been meeting to craft the rules and Fauci expects them to be released "reasonably soon." Upon their release, Fauci says he hopes they will be subject to public comment and receive input from an international consultative conference. The guidelines now cover 15 pathogens and "likely will be modified in the future to include more than the 15." In the past, Fauci has said the release of the guidelines would be necessary before he would support lifting the H5N1 research moratorium.

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Link: https://www.science.org/content/article/us-infectious-disease-chief-urges-flu-scientists-engage-support-h5n1-research

BUSINESSHEALTH CAREHEALTH

Chinese Covid-19 Gene Data That Could Have Aided Pandemic Research Removed From NIH Database

Researcher says he recovered gene sequences after a Chinese scientist asked that they be removed from government archive

By Amy Dockser Marcus Follow , *Betsy McKay* Follow *and Drew Hinshaw* Follow

Updated June 23, 2021 6:24 pm ET

Chinese researchers directed the U.S. National Institutes of Health to delete gene sequences of early Covid-19 cases from a key scientific database, raising concerns that scientists studying the origin of the pandemic may lack access to key pieces of information.

The NIH confirmed that it deleted the sequences after receiving a request from a Chinese researcher who had submitted them three months earlier.

"Submitting investigators hold the rights to their data and can request withdrawal of the data," the NIH said in a statement.

The removal of the sequencing data is described in a new paper posted online Tuesday by Jesse Bloom, a virologist at the Fred Hutchinson Cancer Research Center in Seattle. The paper, which hasn't been peer reviewed, says the missing data include sequences from virus

samples collected in the Chinese city of Wuhan in January and February of 2020 from patients hospitalized with or suspected of having Covid-19.

Some of the deleted information is still available in a paper that was published in a specialized journal, but scientists typically look for gene sequences in major databases like the one the NIH maintains, Dr. Bloom said. Dr. Bloom said he was able to find the deleted data after searching for it elsewhere online.

The missing sequences are unlikely to change researchers' current understanding of the early weeks of the Covid-19 pandemic in Wuhan. But Dr. Bloom said their removal sows doubts about China's transparency in the continuing investigation into the origin of the pandemic.

Some other scientists agreed.

"It makes us wonder if there are other sequences like these that have been purged," said Vaughn S. Cooper, a University of Pittsburgh evolutionary biologist who wasn't involved in the new paper and said he hasn't studied the deleted sequences himself.

To pursue the origin of the pandemic, scientists need access to information that could shed light on how the virus emerged into the human population and began spreading. The removal of information from a database can make it harder for them to find it, potentially slowing their research, as can lack of access to other research. An international team led by the World Health Organization as well as other scientists are investigating how the pandemic began.

According to the NIH statement, the scientist who submitted the sequences requested in June 2020 that they be deleted because they had been updated and were to be posted to another, unspecified database. The investigator said they wanted the older version to be removed to avoid confusion, according to the NIH.

Chinese researchers initially submitted the sequences to the NIH database in March 2020 and published information about them in a paper on a preprint server, according to the NIH. The paper described the use of an advanced sequencing technology to detect SARS-CoV-2, the virus that causes Covid-19. The researchers didn't immediately respond to a request for comment.

China's National Health Commission didn't immediately respond to a request for comment.

One challenge for scientists studying the origin of the virus is the paucity of data from early cases in Wuhan, Dr. Bloom says in the paper. Those data, he says, are mostly limited to virus

sequences obtained in December 2019 from a dozen patients connected to the city's Huanan Seafood Market, the site of the first known outbreak of Covid-19, and a small additional number of sequences collected before late January 2020.

The removal of the sequences yielded "a somewhat skewed picture of viruses circulating in Wuhan early on," Dr. Bloom said. "It suggests possibly one reason why we haven't seen more of these sequences is perhaps there hasn't been a wholehearted effort to get them out there."

The publication of Dr. Bloom's paper could reinforce calls for greater collaboration from China in the global effort to pinpoint the source of SARS-CoV-2.

A WHO official working with the international team that prepared the organization's March report on the origins of the virus said Dr. Bloom's paper didn't radically alter the team's understanding of the early pandemic but did bolster the case for more analysis of the earliest Covid-19 infections.

Dr. Bloom is a co-author of a letter published in May in the journal Science that criticized the WHO report and called for a deeper investigation into two leading hypotheses of the origin of Covid-19: that the pandemic virus entered the human population after escaping from a lab, or that it jumped to humans naturally from infected animals.

He said he realized that sequences had been removed from NIH's Sequence Read Archive database when he read an analysis by other investigators and tried to find the sequences himself.

Following the discovery, he spent mornings and weekends scouring the internet for other sources of the deleted sequences—and ultimately was able to obtain and download them. Dr. Bloom then contacted the NIH to ask why the sequences were removed.

Dr. Cooper, the University of Pittsburgh virologist, said the deleted sequences don't resolve a continuing debate over whether the pandemic emerged from a lab accident or animal spillover into humans. "You could still argue it both ways," he said.

But Dr. Bloom's paper suggests that other early sequence data might still emerge, said Sergei Pond, a Temple University biology professor with expertise on the evolution of viral pathogens.

"If more sequences came to light, especially from early time points, or archival samples elsewhere, everything could change once again," he said. "I think this is likely to happen."

Stephen Goldstein, a University of Utah evolutionary virologist who wasn't involved in Dr. Bloom's research, said it was unclear if any new insights could be gleaned from the deleted sequences. "From a scientific standpoint, I don't think they point to anything nefarious," he said, adding that he had not made his own analysis of the sequences.

The deleted sequences are fragments, and "it's the full genome sequences that have typically been the most informative," said Joel Wertheim, an evolutionary biologist at the University of California, San Diego and an author of a recent paper on the early pandemic.

Dr. Bloom says in his paper that even if there is no further international investigation, the approach he took could be used to learn more about the origin or early spread of the coronavirus.

"We really need to look hard and see if there is other early information about sequences that hasn't been found," he said. "I intend to go through every early preprint I can find about SARS-CoV-2 and see if it describes any data that isn't in the databases."

—Jeremy Page
contributed to this article.

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https://www.wsj.com/articles/after-covid-19-data-is-deleted-nih-reviews-how-its-gene-archive-is-handled-11631545490

WSJ NEWS EXCLUSIVE HEALTH

After Covid-19 Data Is Deleted, NIH Reviews How Its Gene Archive Is Handled

Removal of coronavirus gene sequences that might hold clues to the pandemic's origin sparked concern among scientists and U.S. senators

By Amy Dockser Marcus Follow *and Drew Hinshaw* Follow

Sept. 13, 2021 11:04 am ET

The National Institutes of Health said it was reviewing the removal of genetic data about the Covid-19 virus from an agency-run archive after a scientist raised concerns about the episode earlier this summer.

The data—a series of gene sequences from coronavirus samples obtained from Covid-19 patients in Wuhan in January and February 2020—could hold clues about the origin of the pandemic. The sequences were deleted from the Sequence Read Archive (SRA) last year at the request of one of the Wuhan University researchers who had originally provided them—a move that three Republican U.S. senators questioned in June in a sternly worded letter to NIH Director Francis Collins.

"The efforts by Chinese researchers to delete the data demands additional explanation," Senators Marsha Blackburn (R., Tenn.), Charles E. Grassley (R., Iowa) and Roger Marshall (R.,

Kansas), wrote in the letter. The senators cited as the reason for their inquiry a June 23 Wall Street Journal article about the deletion of the sequences.

In a reply to the senators dated Sept. 8, Dr. Collins said a review was under way to determine "whether appropriate steps were taken to assess this withdrawal request." An NIH spokeswoman on Sunday said that the review had been completed and that NIH leaders would weigh the findings.

"After all the American people have been through since the pandemic started, they deserve straight answers to basic questions which the Biden administration has failed to give them to date," the three senators said on Sunday in a statement after receiving Dr. Collins's letter. The NIH and its parent agency, the Department of Health and Human Services, "have failed to be fully transparent with Congress and the American people," the senators said.

Aides to all three senators said they intended to seek greater clarity from the NIH on its decision to comply with the request and whether that request was handled appropriately.

The agency is withholding the names of the individuals involved in the data's removal to protect their privacy, the NIH spokeswoman said.

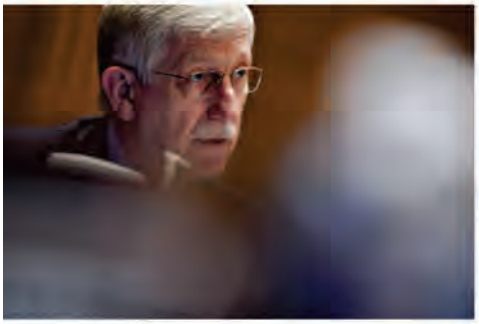
The exchange of letters comes as scientists and the federal government work to determine the origin of the pandemic while criticizing China for withholding information that might be helpful.

An international scientific team led by the World Health Organization reported in March that the pandemic virus, SARS-CoV-2, likely spread to humans by contact with an unknown animal that had been infected by another animal, possibly a bat. But that finding has been sharply criticized in recent months, with some scientists saying there isn't enough evidence to determine whether that hypothesis or the other leading one—that the deadly virus began spreading in humans after escaping from a lab—is the correct one.

U.S. intelligence agencies recently delivered a report to President Biden saying a lack of data made it difficult to reach a definitive conclusion on the origin of the pandemic.

Scientists routinely scour gene sequences of the sort removed from the archive as a way to find clues about the origin and evolution of viral pathogens. Gene sequences often mutate as a virus spreads from person to person, and studying the mutations can shed light on when, where and how pathogens like the Covid-19 virus get their start.

The controversy began in June, when a virologist at the Fred Hutchinson Cancer Research Center in Seattle, Wash., reported in a paper posted online that he had discovered that the sequences had been deleted from the NIH-run database, which is widely used by scientists around the world. As a result, "nobody was aware these sequences existed," the virologist, Jesse Bloom, wrote in the paper, adding that the deletion "suggests a less than wholehearted effort to maximize information about viral sequences from early in the Wuhan epidemic."



Two weeks after Dr. Bloom's paper was posted, the Chinese researchers uploaded the deleted sequences to a public database maintained by the China National Center for Bioinformation. The researchers published information about the sequences in a scientific journal in June 2020.

The researchers didn't respond to an email requesting comment.

China's National Health Commission said the request for the deletion came about as the result of a misunderstanding between the Chinese researchers and the journal that had published the paper describing the sequences, according to an online post identified as that of an employee of the state-affiliated Xinhua News Agency. The commission's vice-minister said that Dr. Bloom had "made up a conspiracy theory that it was a cover up" and that the deleted sequences were of little value for tracing the origin of the pandemic virus, according to a translation in the post.

China's National Health Commission didn't respond to a request for comment.

Dr. Collins said in his letter that the Wuhan University researcher requested the withdrawal of the sequences because updated data was being uploaded to another database and the

researchers wanted to prevent confusion. Dr. Bloom said he later analyzed the sequences in the Chinese database and found them to be identical to the ones removed from the U.S. database.

"To me anyway, it seems like the policies might have ended up being abused to obscure the existence of these sequences," Dr. Bloom said.

It is unusual for data submitted to the Sequence Read Archive to be deleted later on. From March 2020 to March 2021, the archive received about 2.4 million submissions of sequence data, according to a spokeswoman for the National Center for Biotechnology Information, the NIH division that maintains the archive. In that same period, 2.09% of the submissions were updated and 0.19% were withdrawn, the spokeswoman said.

NCBI officials said that it was hard to determine the validity of requests to update or remove data from the Sequence Read Archive and that they take such requests at face value. "We can't adjudicate the truth," said Stephen Sherry, acting director of the NCBI.

The NIH said it retains withdrawn data for the scientific record and in case of disaster recovery.

The review encompassed the archive's procedures and training practices generally as well as the specific request from the Chinese researcher to remove the sequences from the Covid-19 virus, according to Dr. Sherry.

The NIH spokeswoman said the agency is still discussing whether policy changes are needed. "In the meantime," she added, "should the owners of the original data wish to redeposit the SARS-CoV-2 sequences into SRA, we will make that data available."

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