Biosafety and Biosecurity in the Realm of Dual-Use Research of Concern

Joseph Kanabrocki

ABSTRACT

Dual-use potential is commonplace in life sciences research because reagents, experimental approaches and derived knowledge often have the potential to be misused and misapplied to obtain nefarious outcomes (13, 18). Life sciences dual-use research of concern (DURC) presents challenges in two primary realms: Biosafety and biosecurity. The distinction between these is often blurred, as biosafety and biosecurity are related but distinct concepts (36). Strategies to mitigate risks from the biosafety perspective differ from those employed to mitigate biosecurity risks. Biosafety focuses on protection of the researcher, their contacts and the environment via accidental release of a pathogen from containment, whether by direct release into the environment or by a laboratory-acquired infection. Conversely, biosecurity focuses on controlling access to pathogens of consequence and on the reliability of the scientists granted this access (thereby reducing the threat of an intentional release of a pathogen) and/or access to sensitive information related to a pathogen's virulence, host-range, transmissibility, resistance to medical countermeasures, and environmental stability, among other things. The science of biosafety, when applied appropriately and rigorously, has a proven track record of successfully containing dangerous pathogens, thereby enabling important scientific progress while at the same time protecting the public, the environment, and the researchers themselves. A number of additional measures to promote biosafety and biosecurity as they relate to DURC include the following: 1) Establishment of ethical and responsible codes of conduct of life sciences research, including awareness of DURC potential and consideration of alternative, less risky experimental approaches; 2) Strengthening of biosafety practices and capabilities internationally; 3) Education and outreach to the public at large, particularly to our youth, about the importance of life sciences research to public health and well-being; and 4) Communication to the public, political leaders, and funding agencies about the rigor being applied to address DURC-related biosafety and biosecurity concerns.

INTRODUCTION

The purpose of this paper is to provide context for deliberating issues related to *dual-use-research of concern (DURC)* from the perspectives of both biosafety and biosecurity. Historical context for accidental releases (failures in biosafety) and intentional releases (e.g. acts of biowarfare or bioterror, failures in biosecurity) will be discussed. Programmatic context will also be presented to facilitate considerations of critical biosafety programmatic elements and to distinguish these from critical biosecurity programmatic elements.

WHY IS RESEARCH ON PATHOGENIC ORGANISMS NECESSARY?

Naturally Emerging and Re-Emerging Pathogenic Microorganisms:

According to a 2002 World Health Organization report, communicable diseases remain a leading cause of death globally. They account for nearly one-third of the world's deaths (1). The global threat of infectious diseases is exacerbated by the emergence of newly identified pathogens, as well as the re-emergence of pathogens with public health significance. In 2004, the U.S. Government Accountability Office reported that between 1973 and 2003 over 36 newly emerging infectious diseases had been identified (2). More recently, two newly emerging corona viruses surfaced, Severe Acute Respiratory Syndrome coronavirus

(SARS CoV) and Middle East Respiratory Syndrome coronavirus (MERS CoV) in 2003 and 2012, respectively (3).

Most recently an association between infection with Zika virus (a long-known mosquito-borne flavivirus) and Guillain-Barré syndrome and microcephaly was reported in Brazil (July, 2015-4,5). Zika virus was first identified in Uganda in 1947 in monkeys through efforts to monitor yellow fever and was later identified in humans in 1952 (6).

BIOLOCIAL WARFARE AND TERRORISM:

Biological warfare and bioterrorism are not modern human experiences. As early as the 6th century BCE crude military acts included the use of corpses to transmit disease to military enemies. In 1346, the Tartars attacked the well-fortified Genoese controlled city of Caffa (modern Feodosija, Ukraine) by catapulting the plague infected corpses of their dead comrades into the city thinking this would create a plague epidemic in the enemy population (7).

During the French and Indian War, the British gave blankets contaminated with the scabs and secretions of smallpox victims to Native American Indians. Not having been previously exposed to smallpox, and thus not having sufficient immunity, thousands of American Indians died (8).

The discovery of microorganisms and the publication of the Koch's postulates (48) and the germ theory of disease (49) led to a more systematic approach to studying diseases causing microorganisms as well as to the first cases of lab acquired infections (19). The developing science of microbiology was incorporated into more organized, state-sponsored programs aimed at developing biological disease-causing agents into military weapons.

During World War II, the Japanese military conducted biological weapons research in occupied Manchuria on mainland China in the infamous Unit 731. They experimented on and killed at least 3,000 Chinese prisoners of war. New research by Japanese and Chinese scholars suggest that as many as 270,000 Chinese civilians may have been killed in biological weapons experiments during WWII (9).

The Biological Weapons Convention (BWC), the first multilateral disarmament treaty banning the development, production and stockpiling of biological weapons of mass destruction, was opened for signature on 10 April 1972. The BWC entered into force on 26 March 1975.

In spite of the BWC, state-sponsored use and development of biological agents as weapons continued. In 1978, Bulgarian dissident writer Georgi Markov was assassinated in London by ricin poisoning. Ricin, which is derived from castor beans and is difficult to detect in the body, was delivered by injection via a miniature pellet by a modified umbrella. The cause of death was unknown until the pellet was discovered upon autopsy (10).

One year later, in 1979, in the town of Sverdlosk (now Yekaterinberg) in Russia, an inadvertent release of weapons *grade Bacillus anthracis* spores upwind of a populated area

resulted in the largest ever epidemic of inhalation anthrax. The former Soviet Union had a robust bioweapons program that employed up to 55,000 people at 18 facilities under an agency known as Biopreparat (11, 12).

In the early 1990s, the United Nations established an inspection program, termed the United Nations Special Commission (UNSCOM), to ensure Iraq's compliance with the United Nations Security Council Resolution 687, which called for the destruction of Iraqi chemical, biological, and missile weapons, and the International Atomic Energy Agency's efforts to eliminate nuclear weapons facilities in Iraq. This effort uncovered a state of the art, Iraqi bioweapons research laboratory, essentially equivalent to biosafety level 4, at Salman Pak (14).

There is little doubt that the development of modern microbiology and associated biotechnological techniques have made it easier for rogue ideological groups and/or individuals to employ biological agents in efforts to terrorize citizenry to obtain political or ideological goals (33).

The Rajneeshee Cult, an Indian religious group, contaminated restaurant salad bars in Oregon in 1984 with *Salmonella enterica* sv. Typhimurium. Approximately 751 citizens were infected. Their motivation was to incapacitate voters in order to win a local election and to seize political control of Dalles and Wasco counties (13).

In the late 1990s in Japan the Aum Shinrikyo Cult sought to establish a theocratic state in Japan and seize control of the Japanese government. In 1995 they disseminated the chemical agent sarin in the Tokyo subway system and later attempted to release anthrax spores (15).

In 1995, Larry Wayne Harris was arrested and detained in Ohio for possessing cultures of *Yersinia pestis,* the causative agent of plague (16). His motivation was to alert Americans to the Iraqi biological warfare threat and to obtain a separate homeland for white people in the United States. However, the government was only able to convict him of mail fraud because at the time there was no law that prohibited the possession of pathogenic organisms. It was this event that led to the enactment of the Select Agent Transfer Regulation in 1997 (17). The initial iteration of this regulation restricted and monitored only the ***transfer*** of "select agent" pathogens and did not require the registration solely for ***possession*** of these pathogens.

Following the September 11, 2001 attacks on the World Trade Center in New York, the impact of the intentional release of pathogenic microorganisms, as well as the threat of an intentional release, was demonstrated vividly during the *Amerithrax* episode. Highly refined, weaponized, spores of *B. anthracis* were released upon an unsuspecting public, resulting in five deaths, the illness of at least 17 U.S. citizens, and an untold economic impact (18).

BIOSAFETY AS A SCIENTIFIC DISCIPLINE:

HISTORICAL PERSPECTIVE:

It might be argued that biosafety was established as a scientific discipline in the late 1800s coincidentally with the establishment of microbiology as a scientific discipline, as exhibited by publication of the "germ theory" by Pasteur in 1884 (49). The isolation and culturing of bacteria by Robert Koch slightly more than ten years later led to the development of what is known today as Koch's Postulates, which established a causal relationship between pathogenic microorganisms and disease. As microbiologists began to systematically study microorganisms, reports and surveys of lab-acquired infections began to appear as early as 1920 (19).

However, it was the development of offensive biological weapons programs by the U.S. military at Fort Detrick in the early 1940s that marks what is more commonly considered the birth of biosafety, with the concepts of risk assessment, containment, occupational health and applied biosafety research advancing biosafety as a scientific discipline. It was during this time (1943-1969) that Arnold G. Wedum, considered by most to be the "Father of Microbiological Safety", began to publish papers on biosafety practices, risk assessments and applied biosafety research projects (20, 21, 22, 23). Among the principles emerging from Wedum's research was that as engineering controls and practices associated with studying pathogenic microorganisms became more fully developed and sophisticated, the rates of lab-acquired infections decreased significantly (23,24).

The National Cancer Institute's *Classification of Etiological Agents on the Basis of Hazard,* published in 1969, is among the first published documents proposing a series of guidelines by which risk assessments and risk mitigation efforts could be realized. This guideline document offered four hazard classes, a paradigm still followed today, as well as guidance on how to perform a risk assessment, the importance of the development of technical competency for laboratory workers and the concepts of physical containment.

In 1971, President Nixon terminated the US offensive biological weapons program and the U.S. signed the Biological Weapons Convention (BWC) in 1972, converting all programs at Fort Detrick into defensive programs only.

Soon thereafter in 1975, with the emergence of molecular biology and the development of recombinant DNA technologies, concerned scientists assembled at a conference in Asilomar, CA to draft guidelines designed to promote the ethical and responsible application of these new technologies by delineating risk assessment processes, a self-governance oversight structure, and defining roles and responsibilities to ensure it did not threaten public health or the environment. The proceedings from this conference evolved to become the first version of the National Institutes of Health (NIH) *Guidelines for Experiments Involving Recombinant DNA Molecules* (NIH Guidelines-25), first published in 1976. These guidelines emphasize two aspects of containment: Physical containment and biological containment, which are based on existence of natural barriers that limit either the infectivity of an agent (pathogen, vector) for specific hosts or the ability of agent to disseminate or survive in the environment. In addressing these concerns, the NIH Guidelines state that "Since these…means of containment are complimentary, different

levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices."

Continuing in this paradigm of self-governance, in 1984 the Centers for Disease Control and Prevention (CDC), together with the NIH, published the first edition of *Biosafety in Microbiological and Biomedical Laboratories (*BMBL-26*),* regarded by many as the "biosafety bible". The most recent edition of this document, the 5th edition, published in 2009, differs from earlier versions in a number of ways. The most significant addition to the BMBL-5th is added guidance on laboratory biosecurity and risk assessment. This document, together with the NIH Guidelines, serve to this day as the basis for the establishment of credible biosafety programs, certainly in the U.S. and, to some extent, globally.

The combined threats to public health resulting from emerging diseases and the potential for a deliberate release of pathogenic microorganisms altered the research agenda for the U.S. In 2003, the NIH National Institutes of Allergy and Infectious Diseases (NIAID) established *Regional Centers of Excellence (RCE) for Biodefense and Emerging Infectious Diseases Research* to serve as regional foci for developing and conducting cutting edge infectious diseases research (27). These centers were created to develop countermeasures to these threats with the development of vaccines, therapeutics, and diagnostics, among other measures. The Department of Homeland Security also provided financial support, funding the construction of infectious diseases research laboratory infrastructure within the U.S. Twelve of these Regional Biocontainment Laboratories, all capable of operation at BSL3 and ABSL3, were constructed, along with two BSL4 and ABSL4 National Biocontainment Laboratories (28). While the construction of these labs provided state of the art infrastructure in which to conduct infectious diseases research, it also raised concerns among some that this rapid expansion of biocontainment infrastructure and capacity, which was also occurring outside of the U.S., would result in an increase in accidents and releases, thereby threatening the health of the same public that the research conducted in these labs is intended to promote and protect (29). Additional concerns have been raised regarding the capacity of institutions operating high containment laboratories to adequately and appropriately maintain these facilities to ensure their operational integrity. While this is certainly true for institutions in developing countries, it is also true for countries in the developed world as funding sources for operation and maintenance of high containment laboratories is lacking (28).

Biosafety Oversight and Regulation:

Aside from the BWC and the Department of Transportation *Interstate Shipment of Etiologic Agents* (DOT 42 CFR Part 72-1957), the first biosafety "regulation" (i.e. required by law) was issued in 1991 by the Occupational Safety and Health Administration (OSHA) in the form of the *Bloodborne Pathogens Rule* (*BBP Standard*- 29 CFR 1910.1030). This Rule mandated the establishment of biosafety practices to minimize exposure risks, required the use of engineering controls and personal protective equipment, required training on hazard recognition and risk mitigation, and mandated the development of occupational medicine programs for the protection of workers with potential exposures to human-derived potentially infectious materials. The safety-related elements introduced to protect

workers with potential exposures to human pathogens were layered on top of what is traditionally referred to as the OSHA hierarchy of controls to chemical hazards and toxic substances which mandates that elimination or substitution is the most desirable exposure control method (when possible), followed by the implementation of engineering controls.

It was not until the *Amerithrax Attacks* in 2001 that a second regulatory requirement, the *2002 Public Health Security and Bioterrorism Preparedness and Response Act* (PL 107-188), was promulgated for life scientists working with certain pathogens and toxins, known as "select agents" that could "pose a threat to human, animal, and plant safety and health", whether by way of accidental or intentional release. This law is directed primarily at compliance in the realm of biosecurity involving these specific agents. It requires the implementation of robust physical security and personnel reliability measures, including training and technical competency in the lab, to ensure that these agents are accessed and/or manipulated only by those with legitimate, beneficial research intent and that these individuals who are granted this access are deemed reliable and competent.

Concerns about Life-Sciences Dual-Use:

A series of scientific publications in the early 2000s brought an era of heightened concern about the potential for misapplication of scientific technological advancements to achieve malevolent outcomes. The "dual-use" dilemma is one with which the nuclear physics community has been dealing with for more than 70 years. However, in the realm of life sciences, advancements in biotechnology represent a "dual-use" dilemma in which the same technologies can be used legitimately for human benefit and misused for bioterrorism.

In 2001, an Australian group engineered a hypervirulent mousepox virus (30). The goal of this research was to aid in rodent control efforts, however it was quickly recognized that this same approach could be misused with other pox viruses, including smallpox. The following year, an investigator at Stoneybrook University synthesized the poliovirus *de novo* via chemical means (31). In 2005, a research team at the CDC reconstructed the virus responsible for the 1918 influenza pandemic, which killed approximately 50 million people (32).

These publications brought the issue of dual-use research (DUR) in the life sciences to prominence and provided the impetus for a 2004 U.S. National Academies of Sciences (NAS) report ***Biotechnology Research in the Age of Terrorism (2004)*** (33). This report articulated seven categories of experimental approaches that had the potential to generate pathogens as well as pathogen-associated information that could misapplied to cause harm. This report also recommended the creation of an advisory board to help the U.S. address challenges posed by DUR. This led to the establishment of the National Science Advisory Board on Biosecurity (NSABB) in 2004. Since that time, the NSABB has addressed numerous topics related to the DUR issue, including providing recommendations for a national oversight framework for a subset of DUR that may present particular concern. This subset has been termed "*dual-use research of concern" (DURC)*, and NSABB has been developing strategies for raising awareness of the DUR issue among life sciences researchers as well as those outside of traditional life sciences disciplines, promoting

international engagement on dual use issues, considering biosecurity concerns associated with synthetic biology and developing strategies to enhance personnel reliability among researchers.

The NSABB has been tasked with the review of manuscripts that have raised dual use concerns and has provided guidance on how to communicate these manuscripts responsibly. This occurred in October, 2011 when the NSABB was asked to consider two papers, both submitted for publication, reporting transmissibility between mammals (ferrets) of highly pathogenic avian influenza (HPAI) H5N1. Specifically, the NSABB was asked whether these papers should be published and, if so, whether the details defining the specific mutations necessary to convey this transmissibility phenotype should be published in full. NSABB initially recommended that the specific details of the mutations critical to this phenotype be redacted from both manuscripts. Revisions to the original manuscripts were subsequently made by each author, including the provision of additional data revealing that the mammalian transmissible viruses were not as virulent as were the parent viruses (34, 35). However, it was ultimately legal requirements associated with freedom of information that required that the manuscripts either be published in full or become classified (36). Given that the details of some of these experiments had already been presented at a public conference and that it is nearly impossible to classify findings after the research is completed, the NSABB ultimately recommended that both manuscripts be published in full.

Following this intensive and controversial debate concerning the publication of those manuscripts, the U.S. Government issued two federal policies for the identification and oversight of life sciences DURC. A federal policy issued in 2012 requires federal funding agencies to examine their research portfolios to identify DURC and to work with investigators to mitigate risks (37). In addition, effective since 2015 an institutional policy outlines the responsibilities of research institutions to review their research, identify any DURC, and mitigate risks (38). Both of these policies apply to research involving a list of 15 pathogens/toxins and same seven categories of experiments outlined in the NAS report on DURC. These policies promote a collaborative approach involving the federal funders of the research and the research institutions conducting the research to identify DURC and to manage any DURC-associated risks.

BIOSAFETY RISK ASSESSMENT:

Any effort to mitigate the risks posed by research activities involving microbial pathogens must begin with a sound assessment of the scientific bases of these risks and benefits. Many of the pathogenic characteristics, such as environmental stability and virulence factors (e.g. lipopolysaccharide, capsule, endotoxin, exotoxin, adherens, catalase, peroxidase, resistance to antimicrobial drugs) are intrinsic to the pathogen itself while other pathogen characteristics influence the host-pathogen interaction, including species tropism (host range), tissue tropism (routes of infection), infectious dose, antigenic variability, modulation of host immune response, and transmissibility/communicability.

A comprehensive risk assessment considering the above pathogen characteristics is an essential first step towards risk mitigation. However, when pathogens are manipulated

using recombinant or synthetic molecular technologies it is important to assess not only the risks attributed to the parental organism, but to also evaluate the impact attributable to alteration of the pathogen characteristics as a result of the experimental manipulation or modification via these molecular techniques. In the United States, the NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH *Guidelines*) provides the rubric by which the risks associated with alteration of a pathogen's nucleic acid are to be assessed. While technically applicable only to institutions receiving NIH funding to conduct research involving recombinant or synthetic nucleic acids, the NIH *Guidelines* are often adhered to voluntarily not only in the United States, but globally, based primarily on the sound science underpinning the NIH.

BIOSAFETY RISK MITIGATION:

1. ELIMINATION OF HAZARD: USE OF SURROGATE OR SUBSTITUTE ORGANISMS.

The U.S. Occupational Health and Safety Administration (OSHA) has long advocated that in the realm of chemical safety, elimination of the hazard or substitution of the hazard with a less hazardous chemical should be the first step in hazard reduction, when possible (39). Depending on the nature and objectives of a given study and the specific pathogen in question, many fundamental aspects of an infectious diseases research endeavor can be addressed through the use of related but attenuated strains or derivatives of the fully virulent pathogen strain. While good science will mandate that a fully virulent strain, which presumably is ultimately the target of the research effort, be tested to confirm validity of data derived from experiments with attenuated strains, much of the preliminary work often can be accomplished using attenuated or surrogate microorganisms. It is important, however, that prior to start of work on an attenuated pathogen, the investigator should ensure that the attenuated strain remains attenuated.

2. ENGINEERING CONTROLS: SECONDARY CONTAINMENT (LABORATORY FACILITIES) (26)

For studies involving pathogenic microorganisms, protection of the researcher, the public and the environment involves the implementation of actions to *contain* the pathogen. This may take the form of either *biological containment* and/or *physical containment. Biological* containment takes advantage of an understanding of the biology, virulence factors (mentioned above) and life cycle of the pathogen in question and may involve the absence of a suitable host or vector, or management in an environment or system in which the pathogen cannot survive outside its intended host.

The primary focus of *physical* containment is to keep the pathogen contained or confined, sequestered from suitable hosts or vectors. Physical containment is provided through a combination of facility design and operation (termed *secondary* containment) as well as the use of engineering controls and personal protective equipment (PPE) to provide protection of the researcher, termed *primary* containment, i.e. containment at the level of the pathogen.

Included among the containment features designed and constructed into containment laboratories are engineering controls such as effluent decontamination (kill tanks), high-efficiency particulate air (HEPA) filtration of exhaust air and supplied air laboratory suits [Biosafety Level 4 (BSL4)/Animal Biosafety Level 4-(ABSL4)], directional airflow and double door-entry, i.e. *anteroom* (to maintain containment in the event of an accidental spill), as well as architectural and construction parameters designed to facilitate laboratory decontamination (e.g. gaseous decontamination approaches such as vaporous hydrogen peroxide or chlorine dioxide) including cleanable surfaces, seamless flooring and ceiling, and sealed penetrations (BSL3/ABSL3).

3. ENGINEERING CONTROLS: PRIMARY CONTAINMENT (SAFETY EQUIPMENT AND PERSONAL PROTECTIVE EQUIPMENT) (26)

In addition to the secondary containment provided by the containment laboratory itself, physical *primary* containment of a pathogen during research activities also relies upon the use of engineering controls, i.e. specialized equipment and personal protective equipment. Included among these engineering controls are biological safety cabinets, down-draft HEPA-filtered necropsy tables, ventilated and HEPA-filtered animal cage rack systems, fume hoods, on-site autoclaves (for on-site destruction of the pathogen-containing waste), gasketed centrifuge rotors and centrifuge tubes, among other engineering controls.

The last line of defense in terms of engineering controls is the use of personal protective equipment (PPE). Depending on the pathogen in question, this may include the use of respiratory protection (HEPA-filtered negative pressure respirators, HEPA-filtered powered air purifying respirators-PAPR), mucous membrane protection including face shields, safety glasses or goggles and face masks, and protection of skin and/or clothing (e.g. gloves, gowns, lab coats, foot coverings).

4. ADMINISTRATIVE CONTROLS: LABORATORY PRACTICES: (26)

The development of sound and rational standard operating practices (or standard operating procedures-SOPs) in the containment laboratory are critical not only for containment, and ultimately for effective research safety, but also contributes to good science. It is important that SOPs are developed by the researcher, with the primary objective to generate defensible data with the appropriate experimental controls, but also with an eye toward protection of the researcher and ultimately, pathogen containment. A dialogue and collaboration between the researcher and the biosafety professional is essential to do this effectively. However, it is not only important that sound and rational standard procedures be developed and documented, it is equally important that all persons expected to abide by these SOPs become technically competent in following these procedures. This requires time and repetition. In this realm, once again substitution is an appropriate approach. That is, it is prudent to require researchers to become technically proficient in following their SOPs by first learning and practicing the procedures using avirulent or attenuated surrogate microorganisms. Researchers should only be granted

permission to work with fully virulent pathogens after first demonstrating technical proficiency using less risky surrogate microorganisms.

Some procedures can be developed and applied equally across the scope of the research portfolio or a given institute or facility, regardless of the pathogen under study. Procedures in this realm can be team taught and can be led by biosafety, research, and/or veterinary staff. Such procedures include entry and exit from containment, donning and doffing of personal protective equipment, spill clean-up, working in a biological safety cabinet, sharps management, the handling, infections and necropsy of experimental animals, and waste packaging and disposal. However, other procedures are explicitly specific to a given research program and can only be taught and exercised by the specific research team, with critical input and involvement of the principal investigator. An excellent example of a program-specific administrative control is to require temporal and spatial separation when studying multiple strains of influenza, with thorough decontamination of work spaces when changing from on strain to another.

5. OCCUPATIONAL MEDICINE/HEALTH WATCH PROTOCOLS (26):

One cannot overstate the importance of a robust occupational medicine program to support researchers engaged in infectious diseases research. It is critical that occupational medicine care providers are cognizant of, and prepared to deal with, potential infections caused by the pathogens under study in a research program. It is equally critical that lab personnel are educated to recognize the signs and symptoms of infection with the virulent form of the pathogen being studied. Not only is it important that lab personnel are trained to recognize these hazards, but the staff should also be aware of personal risk factors that may make them more susceptible to injury or illness. Reporting of exposure events, spills, releases and near misses must be mandatory. This must be a performance expectation. Negative consequences from management toward researchers must never result from reporting "off-normal" situations and events. Rather, punitive action on behalf of management should only be the result of a *failure to report* these events. In the realm of infectious diseases research, it is important that all researchers are cognizant of, and must monitor, their health status vis-à-vis the activities within the containment laboratory. It is also critical that laboratory workers inform their personal physician of the work they do so that when and investigator becomes ill from an apparent microbial infection, consideration of the potential for a lab acquired infection is part of the calculus.

BIOSECURITY THREAT:

The terms *biosafety* and *biosecurity* are often commingled conceptually. In fact, in some languages, there is only one term that refers to both concepts (e.g. biosécurité, in French). In short, biosafety focuses on accidental release of a pathogen from containment, whether by direct release into the environment or by a laboratory-acquired infection. Conversely, biosecurity focuses on controlling access to pathogens and on the reliability of the scientists granted this access (thereby reducing the threat of an intentional release of a pathogen) and/or access to sensitive information related to a pathogen's virulence, host-

range, transmissibility, resistance to medical countermeasures, and environmental stability, among other things.

From the perspective of biosecurity, there are three threat categories in play: (1) *Agent-associated threats* (i.e. physical access to pathogens of consequence); (2) *Information-associated threats* (i.e. access to information that may enable a malevolent actor, whether state sponsored, terror group sponsored or the lone actor with a cause; and (3) *Threats posed by proliferation of research* (i.e., the more laboratories in operation, the less likely that all of them will have sufficiently rigorous biosafety or biosecurity programs)

In the realm of *agent-associated threats*, an accidental release, via release directly into the environment or rather via infection of lab personnel, represents a *biosafety* breach or failure. However, the intentional release or theft of a pathogen of consequence represents a breach or failure in *biosecurity*. Given that pathogens of consequence are readily obtained in nature, it is not likely that a state-sponsored program would have a need to steal a pathogen of consequence. It is more likely that such a theft would involve either a terrorist group/lone actor lacking the technical expertise needed to isolate from nature and propagate a pathogen of consequence or an *insider threat*, that is an authorized member of a research team studying a pathogen of consequence.

In the U.S., research programs conducting studies with Tier 1 Select Agent pathogens (a subset of select agents that are deemed to pose the greatest threat) physical security elements include locked freezer stocks, perimeter access control, internal access control, monitoring via electronic and written access logs (often via biometric technologies), and regular and routine inventory reconciliations.

Tier 1 Select Agent research programs are also required to develop robust personnel reliability programs (44). Methods designed to ensure ongoing reliability must be developed in Tier 1 research programs in addition to the security measures that are already in place for *any* select agent pathogen such as screening research and support staff to ensure appropriate credentials, experience, and FBI/DOJ criminal background checks. Such ongoing reliability measures include implementation of a *two-person rule,* annual performance evaluations and annual personnel reliability interviews.

One programmatic component particularly critical in this realm is occupational medicine. Some institutions include periodic (e.g. annual) psychiatric evaluations as part of the on-going reliability programs (personal communication). Other institutions conduct annual interviews with all staff to gauge their satisfaction with their occupational experience and to learn of staff concerns or complaints. Still other institutions require a written commitment to an ethical code of conduct on behalf of all research and support staff in the Program. This code must be comprehensive and include aspirational elements (codes of ethics), educational/advisory elements (codes of conduct) and enforceable elements (codes of practice) (45). At the operational level, this code includes a commitment to the following: 1) Training and education; 2) Adherence to established procedures, including and especially, occupational medicine and security procedures; 3) Reporting of off-normal

incidents, accidents and exposures, and; 4) Reporting of observed non-compliance or suspicious activity, among other things.

In the U.S., high containment labs conducting studies with select agents are also required to install robust physical security measures. These measures include multiple layers of access controls, often involving relatively sophisticated technologies such as fingerprint or iris scans, intrusion alarms, closed-circuit security camera systems and security personnel.

On the other hand, *information-associated* threats would most likely be associated with a requirement for relatively sophisticated technologies, equipment and facilities. For example, at present, it is unlikely that the approaches taken to generate H5N1 HPAI transmissible among ferrets via the aerosol route could easily be duplicated by a lone actor or terror group without ready access to the sophisticated equipment, facilities and experimental reagents necessary to reproduce these experiments. It is obvious that technological advancements have made it much easier to manipulate, in fact even synthesize de novo, entire genomes of pathogenic microorganisms (31). Given this, the publication of specific mutations required to acquire a given phenotype, does raise concerns, not only that such experiments could be duplicated by malevolent state-sponsored threats, but also by relatively unsophisticated terror groups or lone actors.

RESEARCH ENTERPRISE TRACK RECORD:

Comprehensive and accurate estimates of laboratory acquired infection rates among the laboratory work force are lacking in large part due to the absence of coordinated and robust incident reporting mechanisms. It is estimated that more than 500,000 workers are employed in life sciences laboratories in the U.S. alone (40). The largest survey (sent to 4000 labs with a 50% response rate) of such infections was reported in 1976 by Pike (19). In this survey, Pike reported that between 1935 and 1978, 4,079 lab-acquired infections occurred, with 14% occurring in clinical labs and 59% occurring in a research setting. A more recent survey conducted by Harding and Byers reported that between 1979-2005, 1,141 laboratory-acquired infections occurred, with 24 of these infections resulting in death (41). These data must be considered within the context that robust reporting mechanisms are lacking and that no mandate to report exists.

Among the principles emerging from Wedum's research findings was that the rates of laboratory-acquired infections decreased significantly as engineering controls and practices associated with studying pathogenic microorganisms became more fully developed and sophisticated (23).

In the realm of research activities involving select agent pathogens, a defined reporting mechanism does exist (Select Agent Program Form 3) and the reporting of thefts, losses or releases, which include occupational exposures and/or lab-acquired infections, is mandatory. A 2012 report published by Henkel, Miller and Weyant (42) examined the frequency of thefts, losses or releases in the U.S. Select Agent Program between 2004-2010 and found that among the 10,000 laboratory workers with access to select agents there were no reports of theft, one report of a lost shipment out of 3,412 select agent transfers, and 11 lab-acquired infections (LAI) with no fatalities and no secondary infections. Close

examination of the eleven laboratory-acquired infection cases, three were associated with exempt select agent facilities, which are diagnostic labs that do not routinely deal with select agent pathogens. Staff in these diagnostic labs lack the training and experience required for work at high containment with pathogenic microorganisms. In this same study, seven of the eleven lab-acquired infections occurred in Biosafety Level 2 (BSL2) environments. Again, staff in labs that operate at BSL2 generally lack the training and competence of research staff working at Biosafety Level 3 (BSL3).

However, it is clear from a review of the literature that lab accidents and exposures are generally underreported. It is important to establish reporting mechanisms, including anonymous reporting pathways, to better catalog and document personnel exposures and/or releases from containment. When reported, investigations should evolve a root cause analysis and lessons learned that should be shared not only locally, but also across the research enterprise. Punitive measures should not be employed for the reporting of lab incidents, but rather should be associated only with a failure to report.

THREATS POSED BY PROLIFERATION OF RESEARCH:

As we have discussed, there has been an expansion of research involving pathogens of consequence, not only in the U.S. but globally, as well as an increase in laboratory capacity to conduct this research. However, international standards on how these facilities are built and designed to operate are lacking. Valid concerns have also been raised that this proliferation of activity and capacity has occurred without the commensurate investment in efforts aimed training the personnel working and/or supporting these facilities. Training programs designed to train not only the scientists who work in these facilities, but also the facility engineering and maintenance personnel charged with operating and maintaining such facilities are under-resourced. For the research staff, this training must include training on risk assessment to ensure that standard procedures developed and deployed are effective at mitigating risks. Additionally, the demonstration of technical competency, developed and honed by first practicing the standard procedures using non-pathogenic surrogate or attenuated microorganisms, must become integral to training programs developed for containment laboratories. Finally, the maintenance and operation of containment facilities, which are costly to construct in the first place, require a substantial financial commitment to ensure effective and safe operation.

In the summer 2014, a number of mishaps in U.S. government labs not only alarmed the public but also compromised the trust and confidence held by political leadership that important research on pathogenic microorganisms could be conducted safely. In each case, the failures in these federal labs, each essentially characterized as accidental releases, were failures rooted in human error, involving either inaccurate risk assessments, lack of appropriate training, failures to maintain accurate pathogen inventories and/or failures in timely reporting of off-normal laboratory incidents. Fortunately, unlike the release of anthrax spores from the former Soviet lab located at Sverdlosk that resulted in citizen infections and deaths, the recent mishaps in federal labs resulted in no infections, neither of lab staff nor the public. Ironically, these incidents led to a moratorium on *gain-of-function* research involving influenza, SARS and MERS, even though the incidents in these labs were not associated in any way with the *gain-of-function* aspects of the research.

The impact of proliferation on biosecurity is obvious. As discussed, the conduct of high containment infectious diseases research requires a commitment to funding, training and education, infrastructure maintenance and responsible science. As the number of facilities supporting this research increases, the likelihood of release to the public and environment also increases. Each facility must develop mechanisms to properly screen research and support staff to ensure appropriate credentials, experience and background. Rational physical security measures including components similar to those described above are also needed to ensure that those with malevolent intentions do not easily gain access to pathogens.

IMPACT OF DUAL-USE POTENTIAL ON BIOSAFETY AND BIOSECURITY

When considering the risks posed by DURC on biosafety, it is clear that that comprehensive biosafety programs, including the elements described above, deployed in appropriately designed, built and maintained containment facilities are effective in facilitating the safe and responsible conduct of research. This is true for any studies involving pathogenic microorganism, including research that poses dual-use concerns. I would argue that the impact of DURC research on biosafety is minimal, provided that a given biosafety program is comprehensive and robust. The potential for a rogue scientist or terrorist to infiltrate a legitimate research program presents a biosecurity threat in the form of pathogen theft and/or intentional release.

The risks posed by publication of information deemed to be DURC would initially appear to increase a risk from state-sponsored agents of bioterror or biowarfare (as opposed to a rogue or a lone actor) given that the technologies required to conduct these types of experiments remain relatively sophisticated. However, as biotechnological advancements continue, it is easier for rogue or lone actors to acquire the tools necessary to use biotechnology for malevolent purposes.

The sub-set of DURC that has generated the most controversy includes *gain-of-function* (GOF) studies involving pathogens with pandemic potential. *Gain-of-function* is a term used to refer to any modification of a biological agent that confers new or enhanced activity. GOF—as well as loss-of-function—studies are commonplace in the life sciences and have been essential elements of modern molecular biology.

The potential benefits of GOF studies include insights to fundamental aspects of host-pathogen interactions, the pandemic potential of emerging pathogens, public health and preparedness efforts, and development of medical countermeasures (46).

The potential risks of GOF studies include the generation of novel engineered pathogens that could pose a pandemic threat accidentally or if released intentionally, as well as the generation of information that could be misused to threaten public health or national security (46). The additional risk of proliferation, discussed above, exacerbates these risks as an the number of labs conducting high containment research increases, including those studies employing GOF approaches. The increasing number of these types of labs also increases the risks of accidental or deliberate release of the experimental pathogen.

In October 2014, the U.S. Government announced a deliberative process involving the NSABB to re-evaluate the potential risks and benefits associated with GOF research involving pathogens with pandemic potential. This process was accompanied by a funding pause for certain GOF studies — those anticipated to generate influenza, MERS, or SARS viruses with enhanced pathogenicity and/or transmissibility in mammals via the respiratory route.

A central NSABB finding derived from this deliberative process was that "*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."* Based upon this finding, the NSABB recommended that "*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."* (47).

The NSABB articulates two criteria for the identification of GOFROC in the report. 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 <u>both</u> of the following attributes:

1. The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.
2. The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.

The report also identifies eight principles that should be considered during the pre-funding evaluation and met before a research proposal involving GOFROC would be considered acceptable for funding:

1. 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.
2. The pathogen that is anticipated to be generated must be judged, based on scientific evidence, to be **able to arise by natural processes**.
3. 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**.
4. 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.
5. 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.
6. 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.

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 project.
8. The proposed research is **ethically justifiable**.

RISK MITIGATION STRATEGIES FOR LIFE-SCIENCES DURC

As a voting member of the NSABB during this deliberative process, I am in agreement with these recommendations. In my view, these recommendations restrict only a very small subset of the important DURC research activities, specifically GOF studies involving pathogens with pandemic potential, thereby facilitating continuation of this important research while simultaneously providing the additional scrutiny and oversight of this research to ensure protection of the public.

When considering solutions to the challenges posed by DURC, it is vital to be mindful of the nature of the specific threat we are trying to mitigate (biosafety vs. biosecurity), as the solutions to each are different.

As already stated, it is clear that that comprehensive biosafety programs, including the elements described above, deployed in appropriately designed, built and maintained containment facilities are effective in facilitating the safe and responsible conduct of research.

Efforts to promote biosafety and biosecurity are vital and must include training and education programs for scientists and support staff (including facility engineers and animal care technicians), as well as funding for infrastructure maintenance, which is particularly important in developing countries.

Additional measures to be taken when conducting DURC experiments, including GOFROC, should include the following:

1. Enhance biosafety practices or features, as warranted, given the specific strains and proposed manipulations
2. Enhance security measures around strains, reagents, notebooks, and personnel
3. Prohibit certain additional GOFROC experiments without prior approval
4. Treat the research as if it is subject to the federal DURC policies, even if it is not already
5. Identify certain experimental outcomes that would trigger a re-evaluation of the risks and benefits prior to proceeding with a study
6. Communicate regularly and coordinate with federal, state, and local public health and safety officials on accident and theft response
7. Undertake broad efforts to strengthen laboratory biosafety and biosecurity and seek to raise awareness about the specific issues associated with GOF research of concern
8. Engage the international community in dialogue about the oversight and responsible conduct of GOF research of concern

However, I believe that a number of additional measures to promote DURC-related biosafety and biosecurity should include the following:

1. Establishment of ethical and responsible codes of conduct of life sciences research, including awareness of DURC potential and consideration of alternative, less risky experimental approaches. As discussed above, a fundamental risk mitigation approach is elimination or substitution. Whenever possible, *gain-of-function* research involving pathogens with pandemic potential should include explicit and documented consideration of alternative approaches and/or the use of surrogate or attenuated pathogen strains. Also, it is critical that all research staff involved with these studies are committed to the ethical and responsible conduct of science.
2. Strengthening of biosafety practices and capabilities internationally. Based upon the track record of the infectious diseases research community, it is clear that when appropriate biosafety and biosecurity measures are in place, research with dangerous pathogens can be conducted safely, thereby providing scientific progress that serves to benefit the health and well-being of the public. However, high containment research involves a commitment to on-going financial support and to on-going education and training and to sharing of lessons learned. It is important to establish reporting mechanisms, including anonymous reporting pathways, to better catalog and document personnel exposures and/or releases from containment. When reported, investigations should evolve a root cause analysis and lessons learned that should be shared not only locally, but also across the research enterprise.
3. Education and outreach to the public at large, particularly to our youth, about the importance of life sciences research to public health and well-being.
4. Raising awareness of DURC among scientists.
5. Development of mechanisms for incident reporting and sharing of best practices.
6. Communication to the public, political leaders, and funding agencies about the rigor being applied to address DURC-related biosafety and biosecurity concerns.

REFERENCES

1. The World Health Organization (2002) The World Health Report: Reducing Risks, Promoting Health Life (http://www.who.int/whr/2002/en/whr02_en.pdf)

2. The United States Government Accountability Office (2004) Emerging Infectious Diseases: Review of State and Federal Disease Surveillance Efforts (GAO-04-877 Disease Surveillance, http://www.gao.gov/new.items/d04877.pdf)

3. de Wit E, van Doremalen N, Falzarano D, Munster VJ., *SARS and MERS: recent insights into emerging coronaviruses*. Nat Rev Microbiol. 2016 Aug;14(8):523-34. doi: 10.1038/nrmicro.2016.81.

4. Campos GS, Bandeira AC, Sardi SI, *Zika Virus Outbreak, Bahia, Brazil*. Emerg Infect Dis. 2015 Oct;21(10):1885-6. doi: 10.3201/eid2110.150847.

5. Pan American Health Organization. Epidemiological alert. *Increase in microcephaly in the northeast of Brazil—epidemiological alert.* Washington DC: World Health Organization, Pan American Health Organization; 2015. http://www.paho.org/hq/index.php?

6. Dick GW, Kitchen SF, Haddow AJ. *Zika virus. I. Isolations and serological specificity*. Trans R Soc Trop Med Hyg 1952;46:509–20.

7. Wheelis, Mark. *Biological Warfare at the 1346 Siege of Caffa*. 2002. Emerging Infectious Diseases 8(9):971-975.

8. Waldman, Carl, *Atlas of the North American Indian*, Published by Facts on File, New York/ Oxford UK, 1985, ISBN 10: 0871968509/ ISBN 13: 9780871968500

9. David C. Rapoport. In James M. Ludes, Henry Sokolski (eds.), *Terrorism and Weapons of the Apocalypse*, *Twenty-First Century Weapons Proliferation: Are We Ready?* Routledge, 2001. pp. 19, 29

10. Papaloucas M, Papaloucas C, Stergioulas A., *Ricin and the assassination of Georgi Markov*. Pak J Biol Sci. 2008 Oct 1;11 (19):2370-1.

11. Meselson M, Guillemin J, Hugh-Jones M, et al. *The Sverdlovsk anthrax outbreak of 1979,* (November 1994). Science. **266** (5188): 1202 8. Doi: 10.1126/science.7973702. PMID 7973702

12. Ken Alibek and S. Handelman. *Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World - Told from Inside by the Man Who Ran it*. 1999. Delta (2000) ISBN 0-385-33496-6 (1)

13. Jacobs MK, *The history of biologic warfare and bioterrorism*. Dermatol Clin. 2004

Jul;22(3):231-46, v. PMID:15207305, DOI: 10.1016/j.det.2004.03.008

14. Barton R, *The application of the UNSCOM experience to international biological arms control.* United Nations Special Commission. Crit Rev Microbiol. 1998;24(3):219-33. PMID: 9800101, DOI: 10.1080/10408419891294253

15. Keim P, Smith KL, Keys C. Takahashi H. Kurata T, Kaufmann A, J, *Molecular investigation of the Aum Shinrikyo anthrax release in Kameido, Japan*. Clin Microbiol.., 2001 Dec;39(12):4566-7. PMID: 11724885, PMCID: PMC88589, DOI: 10.1128/JCM.39.12.4566-4567.2001

16. Jessica Eve Stern, *Larry Wayne Harris: The Talkative Terrorist,* Published in Jonathan Tucker, ed., Toxic Terror (Cambridge: MIT Press, 2000)

17. *Additional Requirements for Facilities Transferring or Receiving Select Agents*, 42 CFR Part 72.6; effective April 15, 1997

18. *AMERITHRAX INVESTIGATIVE SUMMARY,* The United States Department of Justice, Released Pursuant to the Freedom of Information Act, Friday, February 19, 2010

19. Pike RM. *Laboratory-associated infections: summary and analysis of 3921 cases*. Health Lab Sci **1976**; 13:105–14.

20. Wedum AG, Barkley WE, Hellman A. *Handling of infectious agents*. J Am Vet Med Assoc. 1972 Dec 1;161(11):1557-67. PMID: 4565075

21. Kruse RH, Wedum AG. *Cross infection with eighteen pathogens among caged laboratory animals.* Lab Anim Care. 1970 Jun; 20(3):541-60. PMID: 4317391

22. Glick CA, Wedum AG. *Lead tests by high-velocity impact of infectious specimen containers*. Public Health Rep. 1969 Sep;84(9):783-6. PMID: 4979878

23. Wedum AG. II. *Airborne Infection in the Laboratory*. Am J Public Health Nations Health. 1964 Oct;54:1669-73. PMID: 14240489

24. Wedum AG. *Laboratory Safety in Research with Infectious Aerosols.* Public Health Rep. 1964 Jul; 79:619-33. PMID: 14177793

25. Wivel Nelson A. *Historical Perspectives Pertaining to the NIH Recombinant DNA Advisory Committee*. Human Gene Therapy. January 2014, 25(1): 19-24. doi:10.1089/hum.2013.2524. Published in Volume: 25 Issue 1: January 20, 2014

26. *Biosafety in Microbiological and Biomedical Laboratories 5th Edition,* U.S. Department of Health and Human Services Public Health Service Centers for Disease Control and Prevention National Institutes of Health HHS Publication No. (CDC) 21-1112 Revised December 2009

27. Kanabrocki J, Stanley Jr. S, *The Midwest Center of Excellence for Biodefense and Emerging Infectious Diseases Research (MRCE) Biosafety Training Program: An Integrated Approach to Biosafety Education,* 2007, *Applied Biosafety* Vol. 12, No. 4

28. Kanabrocki J. *Interactions between biocontainment laboratories and their communities: a successful work in progress*. Trends in Microbiology May 2011, Vol. 19, No. 5

29. Casadevall, A., and M. Imperiale, 2014 *Risks and benefits of gain-of-function experiments with pathogens of pandemic potential, such as influenza virus*- mBio 5 e01730-14

30. Jackson RJ, Ramsay AJ, Christensen CD, Beaton S, Hall DF, Ramshaw IA.*Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox*. J Virol. 2001 Feb; 75(3):1205-10.

31. Cello J, Paul AV, Wimmer E. *Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template*. Science. 2002 Aug 9; 297(5583): 1016-8. Epub 2002 Jul 11.

32. Tumpey TM, et al. 2005. *Characterization of the reconstructed 1918 Spanish influenza pandemic virus.* Science 310:77–80

33. *Biotechnology Research in an Age of Terrorism.* Editors: National Research Council (US) Committee on Research Standards and Practices to Prevent the Destructive Application of Biotechnology.Washington (DC): National Academies Press (US); 2004.

34. Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, Shinya K, Zhong G, Hanson A, Katsura H, Watanabe S, Li C, Kawakami E, Yamada S, Kiso M, Suzuki Y, Maher EA, Neumann G, Kawaoka Y. *Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets*. Nature. 2012 May 2;486(7403):420-8. doi: 10.1038/nature10831.

35. Herfst S, Schrauwen EJ, Linster M, Chutinimitkul S, de Wit E, Munster VJ, Sorrell EM, Bestebroer TM, Burke DF, Smith DJ, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. *Airborne transmission of influenza A/H5N1 virus between ferrets.* Science. 2012 Jun 22; 336 (6088):1534-41. doi: 10.1126/science.1213362. PMID: 22723413

36. Gronvall, G., 2013 H5N1- *A case study for dual-use research.* Council on Foreign Relations.

37. United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern. March 29, 2012 http://osp.od.nih.gov/sites/default/files/resources/United_States_Government_Policy_for_Oversight_of_DURC_FINAL_version_032812_1.pdf

38. United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. Release date: September 24, 2014 Effective date: September 24, 2015. https://www.phe.gov/s3/dualuse/documents/durc-policy.pdf

39. Occupational Health and Safety Administration (OSHA). *Basics of Informed Substitution and Alternatives Assessment, Transitioning to Safer Chemicals: A Toolkit for Employers and Workers.* https://www.osha.gov/dsg/safer_chemicals/basics.html

40. Sewell DL. *Laboratory associated infections and biosafety*. Clin Microbiol Rev **1995**; 8:389–405.

41. Harding AL, Byers KB. *Epidemiology of laboratory-associated infections*. In: Fleming DO, Hunt DL, eds. Biological safety: principles and practices. 3rd ed. Washington, DC: ASM Press, **2000**:35–54.

42. Henkel R, Miller T, Weyant R. *Monitoring Select Agent Theft, Loss and Release Reports in the United States-2004-2010,* 2012 Applied Biosafety Vol. 17, No. 4

43. Lemon, S. *Managing Personnel Reliability at the Galveston National Laboratory University of Texas Medical Branch*

44. Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins Animal and Plant Health Inspection Service (APHIS) *Guidance for Suitability Assessments 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73* July 8, 2013

45. Rohde C, Smith D, Martin D, Fritze D and Stalpers J. *Code of Conduct on Biosecurity for Biological Resource Centres: procedural implementation*. International Journal of Systematic and Evolutionary Microbiology (2013), 63, 2374–2382 DOI 10.1099/ijs.0.051961-0

46. Casadevall, A., and M. Imperiale, 2014 Risks and benefits of gain-of-function experiments with pathogens of pandemic potential, such as influenza virus- mBio 5 e01730-14

47. National Sciences Advisory Board on Biosecurity, May 24, 2016. *Recommendations for the Evaluation and Oversight or Proposed Gain-of-Function Research*

48. Koch, Robert. *Investigations into the Etiology of Traumatic Infective Diseases.* The New Sydenham Society, 1880.

49. Pasteur, Louis (1880) [May 1880]. "(translated from French)" [On the extension of the germ theory to the etiology of certain common diseases]. *Comptes rendus, de l'Academie des Sciences*. **XC**. Ernst, H.C. (trans). pp. 1033–44.