Section I—Introduction

Biosafety in Microbiological and Biomedical Laboratories (BMBL) has become the overarching guidance document for the practice of biosafety in the U.S.—the mechanism for addressing the safe handling and containment of infectious microorganisms and hazardous biological materials. The principles of biosafety introduced in 1984 in the first edition of BMBL[1] and carried through this edition remain steadfast. These principles are containment and risk assessment. The fundamentals of containment include the 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. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can help prevent Laboratory-associated infections (LAI). The purpose of periodic updates of BMBL is to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health. In this way, the guidance provided within the BMBL will continue to serve the microbiological and biomedical community as a relevant and valuable reference.

The uncertainty and change regarding the identification of emerging agents and the requirements for containment and safe storage of pathogens continues to accelerate since the last edition of the BMBL was published. New infectious agents and diseases have emerged. Work with infectious agents in public and private research, public health, clinical and diagnostic laboratories, and in animal care facilities has expanded. World events have demonstrated new threats of bioterrorism. For these reasons, organizations and laboratory directors are compelled to evaluate and ensure the effectiveness of their biosafety programs, the proficiency of their workers, as well as the capability of equipment, facilities, and management practices to provide containment and security of microbiological agents. Similarly, individual workers who handle pathogenic microorganisms must understand the containment conditions under which infectious agents can be safely manipulated and secured. Application of this knowledge and the use of appropriate techniques and equipment will enable the microbiological and biomedical community to help prevent personal, laboratory, and environmental exposure to potentially infectious agents or biohazards.

The Occurrence of Laboratory-associated Infections

Published reports of LAIs first appeared around the start of the 20th century. By 1978, four studies by Pike and Sulkin collectively identified 4,079 LAIs resulting in 168 deaths occurring between 1930 and 1978.[2–5] These studies found that the ten most common causative agents of overt infections among workers were *Brucella* spp., *Coxiella burnetii*, hepatitis B virus (HBV), *Salmonella enterica* serotype Typhi, *Francisella tularensis*, *Mycobacterium tuberculosis*, *Blastomyces*

dermatitidis, Venezuelan equine encephalitis virus, *Chlamydia psittaci*, and *Coccidioides immitis*. The authors acknowledged that the 4,079 cases did not represent all LAIs that occurred during this period, since many laboratories chose not to report overt cases or conduct surveillance programs to identify subclinical or asymptomatic infections.

In addition, historical reports of LAIs seldom provided data sufficient to determine incidence rates, complicating quantitative assessments of risk. Similarly, there were no distinguishable accidents or exposure events identified in more than 80% of the LAIs reported before 1978. Studies did show that, in many cases, the infected person worked with a microbiological agent or was in the vicinity of another person handling an agent.[2–6]

During the 20 years following the Pike and Sulkin publications, a worldwide literature search by Harding and Byers revealed 1,267 overt infections with 22 deaths.[7] Five deaths were of fetuses aborted as the consequence of a maternal LAI. *Mycobacterium tuberculosis*, *Coxiella burnetii*, hantavirus, arboviruses, HBV, *Brucella* spp., *Salmonella* spp., *Shigella* spp., hepatitis C virus, and *Cryptosporidium* spp. accounted for 1,074 of the 1,267 infections. The authors also identified an additional 663 cases that presented as subclinical infections. Like Pike and Sulkin, Harding and Byers reported that only a small number of the LAI involved a documented specific incident. The non-specific associations reported most often by these authors were working with a microbiological agent, being in or around the laboratory, or being around infected animals.

The findings of Harding and Byers indicated that clinical (diagnostic) and research laboratories accounted for 45% and 51%, respectively, of the total LAIs reported. This is a marked difference from the LAIs reported by Pike and Sulkin prior to 1979, which indicated that clinical and research laboratories accounted for 17% and 59%, respectively. The relative increase of LAIs in clinical laboratories may be due in part to improved employee health surveillance programs that are able to detect subclinical infections, or to the use of inadequate containment procedures during the early stages of culture identification.

Comparison of the more recent LAIs reported by Harding and Byers with those reported by Pike and Sulkin suggests that the number is decreasing. Harding and Byers note that improvements in containment equipment, engineering controls, and greater emphasis on safety training may be contributing factors to the apparent reduction in LAIs over two decades. However, due to the lack of information on the actual numbers of infections and the population at risk, it is difficult to determine the true incidence of LAIs.

Publication of the occurrence of LAIs provides an invaluable resource for the microbiological and biomedical community. For example, one report of occupational exposures associated with *Brucella melitensis*, an organism capable of

transmission by the aerosol route, described how a staff member in a clinical microbiology laboratory accidentally sub-cultured *B. melitensis* on the open bench.[8] This error and a breach in containment practices resulted in eight LAIs with *B. melitensis* among 26 laboratory members—an attack rate of 31%.

Reports of LAIs can serve as lessons in the importance of maintaining safe conditions in biomedical and clinical laboratories.

Evolution of National Biosafety Guidelines

National biosafety guidelines evolved from the efforts of the microbiological and biomedical community to promote the use of safe microbiological practices, safety equipment, and facility safeguards that reduce LAIs and protect public health and the environment. The historical accounts of LAIs raised awareness about the hazards of infectious microorganisms and the health risks to laboratory workers who handle them. Many published accounts suggested practices and methods that might prevent LAIs.[9] Arnold G. Wedum was the Director of Industrial Health and Safety at the United States Army Biological Research Laboratories, Fort Detrick, from 1944 to 1969. His pioneering work in biosafety provided the foundation for evaluating the risks of handling infectious microorganisms and for recognizing biological hazards and developing practices, equipment, and facility safeguards for their control. Fort Detrick also advanced the field by aiding the development of biosafety programs at the United States Department of Agriculture (USDA), National Animal Research Center (NARC) and the United States Department of Health and Human Services (DHHS), Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH). These governmental organizations subsequently developed several national biosafety guidelines that preceded the first edition of BMBL.

In 1974, the CDC published *Classification of Etiologic Agents on the Basis of Hazard*.[10] This report introduced the concept for establishing ascending levels of containment that correspond to risks associated with handling infectious microorganisms that present similar hazardous characteristics. Human pathogens were grouped into four classes according to mode of transmission and the severity of disease they caused. A fifth class included non-indigenous animal pathogens whose entry into the United States was restricted by USDA policy.

The NIH published *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* in 1974.[11] These guidelines established three levels of containment based on an assessment of the hypothetical risk of cancer in humans from exposure to animal oncogenic viruses or a suspected human oncogenic virus isolate.[12,13] In 1976, NIH first published the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*.[14] The current *NIH Guidelines* described in detail the microbiological practices, equipment, and facility safeguards that correspond to four ascending levels of physical

containment and established criteria for assigning experiments to a containment level based on an assessment of potential hazards of this continually evolving technology.[15] The evolution of these guidelines set the foundation for developing a code of practice for biosafety in microbiological and biomedical laboratories. Led by the CDC and NIH, a broad collaborative initiative involving scientists, laboratory directors, occupational physicians, epidemiologists, public health officials, and health and safety professionals developed the first edition of BMBL in 1984.[16] The BMBL provided the technical content not previously available in biosafety guidelines by adding summary statements conveying guidance pertinent to infectious microorganisms that had caused LAIs. The sixth edition of BMBL is also the product of a broad collaborative initiative committed to perpetuate the value of this national biosafety code of practice.

Risk Criteria for Establishing Ascending Levels of Containment

The primary risk criteria used to define the four ascending levels of containment, referred to as Biosafety Levels 1 through 4, are infectivity, severity of disease, transmissibility, and the nature of the work being conducted. Another important risk factor for agents that cause moderate to severe disease is the origin of the agent, whether indigenous or exotic. Each level of containment describes the microbiological practices, safety equipment, and facility safeguards for the corresponding level of risk associated with handling an agent. The facility safeguards associated with Biosafety Levels 1 through 4 help protect non-laboratory occupants of the facility, the public health, and the environment.

Biosafety Level 1 (BSL-1) is the basic level of protection and is appropriate for defined and characterized strains of viable biological agents that are not known to cause disease in immunocompetent adult humans. Biosafety Level 2 (BSL-2) is appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. Biosafety Level 3 (BSL-3) is appropriate for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections, and that are indigenous or exotic in origin. Exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available are restricted to high containment laboratories that meet Biosafety Level 4 (BSL-4) guidelines.

It is important to emphasize that the causative incident for most LAIs is unknown.[7,8] Less obvious exposures such as the inhalation of infectious aerosols or direct contact of broken skin or mucous membranes with droplets containing an infectious microorganism or surfaces contaminated by droplets may possibly explain the incident responsible for a number of LAIs. Manipulations of liquid suspensions of microorganisms may produce aerosols and droplets. Small-particle aerosols have respirable size particles that may contain one or several microorganisms. These small particles stay airborne and easily disperse

throughout the laboratory. When inhaled, the human lung will retain these particles. Larger particle droplets rapidly fall out of the air, contaminating gloves, the immediate work area, and the mucous membranes of unprotected workers. A procedure's potential to release microorganisms into the air as aerosols and droplets is the most important operational risk factor that supports the need for containment equipment and facility safeguards.

Agent Summary Statements

The sixth edition, as in all previous editions, includes agent summary statements that describe the hazards, recommended precautions, and levels of containment appropriate for handling specific human and zoonotic pathogens in the laboratory and in facilities that house laboratory vertebrate animals. Agent summary statements are included for agents that meet one or more of the following three criteria:

1. The agent is a proven hazard to laboratory personnel working with infectious materials;
2. The agent is suspected to have a high potential for causing LAIs even though no documented cases exist; and
3. The agent causes grave disease or presents a significant public health hazard.

Scientists, clinicians, and biosafety professionals prepared the statements by assessing the risks of handling the agents using standard protocols followed in many laboratories. **No one should conclude that the absence of an agent summary statement for a human pathogen means that the agent is safe to handle at BSL-1 or without a risk assessment to determine the appropriate level of containment.** Laboratory directors should also conduct independent risk assessments before beginning work with an agent or procedure new to the laboratory, even though an agent summary statement is available. There may be situations where a laboratory director should consider modifying the precautionary measures or recommended practices, equipment, and facility safeguards described in an agent summary statement. In addition, laboratory directors should seek guidance when conducting risk assessments. Knowledgeable colleagues, institutional safety committees, institutional biosafety committees, biosafety officers, and public health, biosafety, and scientific associations are excellent resources.

The agent summary statements in the fifth edition of BMBL were reviewed in the course of preparing the sixth edition. There are new and updated agent summary statements including those for agents classified as Select Agents. For example, there is an updated section on arboviruses and related zoonotic viruses including new agent summary statements as well as statements for recently emerged agents such as Middle East Respiratory Syndrome coronavirus (MERS-CoV).

The sixth edition includes a substantially revised section on risk assessment that emphasizes the critical importance of this process in selecting the appropriate practices and level of containment. That section intentionally follows this introduction because risk assessment is the core principle that supports a code of practice for safe handling of infectious agents in microbiological and biomedical laboratories.

Laboratory Biosecurity

The nation also continues to face a challenge in safeguarding the public health from potential domestic or international bioterrorism. Existing standards and practices may require adaptation to ensure protection from such hostile actions. Federal regulations mandate increased security within the microbiological and biomedical community in order to protect high consequence biological pathogens and toxins from theft, loss, or misuse. The sixth edition of BMBL includes an update on laboratory biosecurity—the discipline addressing the security of microbiological agents and toxins and the threats posed to human and animal health, the environment, and the economy by deliberate misuse or release. A careful review of the laboratory biosecurity concepts and guidelines in Section VI is essential for all laboratory workers.

Using *Biosafety in Microbiological and Biomedical Laboratories*

BMBL is a code of practice and an authoritative reference. Knowledge sufficient to work safely with hazardous microorganisms requires a careful review of multiple sections of the BMBL. This will offer the reader an understanding of the biosafety principles that serve as the basis for the concepts and recommendations included in this reference. Reading only selected sections will not adequately prepare even an experienced laboratory worker to handle potentially infectious agents safely.

The recommended practices, safety equipment, and facility safeguards described in the BMBL are advisory. The intent was and is to establish a voluntary code of practice, one that all members of a laboratory community will together embrace to safeguard themselves and their colleagues, and to protect the public health and environment.

Additional appendices have been added to the sixth edition of the BMBL, including: Appendix K—Inactivation and Verification; Appendix L—Sustainability; Appendix M—Large Scale Biosafety; and Appendix N—Clinical Laboratories. In Appendix K, content has been added on inactivation verification, as recent events have demonstrated that it may be insufficient to follow a published inactivation procedure and assume that it is capable of providing complete inactivation of all pathogenic organisms present in a sample. In Appendix L, content has been added to assist laboratories with finding methods to reduce the significant operating costs associated with laboratories. In Appendix M, biosafety considerations for large-scale production of agents has been added, in recognition of the

interest in the use of biological agents in the generation of biopharmaceuticals. Finally, in Appendix N, content on the safe handling of biological materials in clinical laboratories has been added, as the risk assessment of handling specimens with unconfirmed but suspected high-risk agents can be significantly different from the assessment traditionally generated in microbiology laboratories.

The BMBL should not be used as a single source of biosafety information; it provides the basis for a rational risk assessment to be developed and reviewed by the competent stakeholders at an institution. Inclusion of all relevant stakeholders, including the biosafety office or officer, animal care staff, facilities staff, management, and the Institutional Biosafety Committee, or equivalent resource, is needed to ensure all relevant parties provide input and reach consensus on the risk assessment.

Looking Ahead

Although Laboratory-associated infections are infrequent, it is critical that the microbiological and biomedical communities continue their resolve to remain vigilant and avoid complacency. The widely reported incidents of accidental shipments of or potential exposures to high-consequence pathogens over the last several years demonstrate that accidents and unrecognized exposures continue to occur. The absence of clear evidence of the means of transmission in most documented LAIs should motivate persons at risk to be alert to all potential routes of exposure. The accidental release of microbial aerosols is a probable cause of many LAIs,[17] which demonstrates the importance of worker training and the ability to recognize potential hazards and correct unsafe habits. Attention to and proficient use of work practices, safety equipment, and engineering controls are also essential.

Understanding the principles of biosafety, the use of well-executed risk assessments, and the adherence to the microbiological practices, containment, and facility safeguards described in BMBL will continue to contribute to a safer and healthier working environment for laboratory staff, adjacent personnel, and the community.

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Section II—Biological Risk Assessment

The ongoing practice of biological risk assessment is the foundation of safe laboratory operations. Risk assessment requires careful judgment and is an important responsibility for directors and principal investigators (PI) of microbiological and biomedical laboratories. Institutional leadership and oversight resources, such as Institutional Biosafety Committees (IBCs) or equivalent resources, animal care and use committees, biological safety professionals, occupational health staff, and laboratory animal veterinarians also share in this responsibility. When assessing risk, it is essential to broadly engage stakeholders, including laboratory and facility staff and subject matter experts, in committee reviews of work and discussions of past studies of Laboratory-associated infections (LAIs) and other published research. The biological risk assessment process is used to identify the hazardous characteristics of an infectious or potentially infectious agent or material, if known; the activities that can result in a person's exposure to an agent; the likelihood that such exposure will cause an LAI; and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate mitigations, including the application of Biosafety Levels and good microbiological practices, safety equipment, and facility safeguards that can help prevent LAIs.

Promoting a positive culture of safety by integrating a risk management process into daily laboratory operations results in the ongoing identification of hazards and prioritization of risks and the establishment of risk mitigation protocols tailored to specific situations. To be successful, this process must be collaborative and inclusive of all stakeholders. Further, it must recognize a hierarchy of controls, beginning with the elimination or reduction of hazards, then progress to implementing the appropriate engineering and/or administrative controls to address residual risks, and, if necessary, identifying personal protective equipment (PPE) to protect the worker.[1]

For the purposes of this section, hazards are defined as substances or situations capable of causing adverse effects to health or safety.[2] Risks occur when people interact with hazards and are a function of both the probability of adverse events and expected consequences of a potential incident.[2] The product of probability and consequence estimates provide a relative value that can be used to prioritize risks. Since it is impossible to eliminate all risk, unless the associated hazard is eliminated, the risk assessment evaluates recognized risks associated with a particular hazard and reduces risk to an institutionally acceptable level through a documented process. For the biological laboratory, this process is usually qualitative with classifications from high- to low-risk. This section provides guidance on conducting a risk assessment, implementing a risk mitigation program, communicating during and after the assessment, and developing practices to support ongoing application of the risk assessment process.

Risks are best mitigated by combining and overlapping risk management practices and risk mitigation controls to offer redundant protections for the worker, community, and the environment. Working through the risk assessment process identifies best practices for manipulating biological agents, how to integrate multiple containment or protection strategies, and how to respond if something does not go as planned. When performed comprehensively, it accounts for changing methodologies, procedures, and regulations as the work evolves.

Adverse consequences, like LAIs, are more likely to occur if the risks are unidentified or underestimated. By contrast, imposition of safeguards more rigorous than needed may result in additional expense and burden for the laboratory with little enhancement of laboratory safety. However, where there is insufficient information to make a clear determination of risk, consider the need for additional safeguards until more data are available.

The Risk Management Process

The sixth edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* provides guidance on risk mitigation measures to address common agent and protocol risks. As all possible adverse incidents can't be predicted, judgments and decisions about control measures sometimes need to be based on incomplete information. Special risks, associated with a particular type of laboratory, may require more caution in the risk assessment; for example, clinical laboratories rarely have the benefit of agent information, as they are typically looking to identify the causative agent for a medical diagnosis. Please refer to Appendix N for additional information on clinical laboratories.

This section describes a six-step approach that gives structure to the risk management process and reinforces an ongoing positive culture of safety. Other methodologies may be useful, including the process described in the WHO Laboratory Biosafety Manual.

The initial factors to consider in risk assessment fall into two broad categories: agent hazards and laboratory procedure hazards. Following the assessment of the inherent risk, the Biosafety Level and any additional indicated mitigation strategies are determined. Before implementation of the controls, the risk assessment and selected safeguards should be reviewed with a biosafety professional, subject matter expert, and the IBC or equivalent resource. Then, as part of an ongoing assessment of risk management, the proficiency of staff regarding safe practices and the integrity of safety equipment is evaluated and training or competency gaps are addressed. Finally, the management strategies are revisited regularly to reassess risks and mitigations and are updated when appropriate.

First, identify hazardous characteristics of the agent and perform an assessment of the inherent risk, which is the risk in the absence of mitigating factors. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible host, severity of disease, and the availability of preventive measures and effective treatments. Also consider possible routes of transmission of infection in the laboratory, infectious dose (ID), stability in the environment, host range, whether the agent is indigenous or exotic to the local environment, and the genetic characteristics of the agent.[3–6]

Several excellent resources provide information and guidance for making an initial risk assessment. Section VIII of BMBL provides agent summary statements for many agents that are associated with LAIs or are of increased public concern. Agent summary statements also identify known and suspected routes of transmission of Laboratory-associated infections and, when available, information on infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent. Safety documents from reputable sources are also valuable, such as the Pathogen Data Safety Sheets generated by the Public Health Agency of Canada (PHAC); the Pathogen Data Safety Sheets are available at https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html. A thorough examination of the agent hazards is necessary when the intended use of an agent does not correspond with the general conditions described in the agent summary statement or when an agent summary statement is not available. In addition, it is always helpful to seek guidance from colleagues with experience in handling the agent and from biological safety professionals.

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* has incorporated an agent Risk Group (RG) classification for laboratory use that describes four general Risk Groups based on these principle characteristics and the route of transmission of the natural disease; this list is found in Appendix B of the *NIH Guidelines*. ABSA International also has a compendium of organisms and Risk Group assignments from several countries and organizations available at https://my.absa.org/Riskgroups. Agent Risk Group assignments assist with an initial estimate of the pathogen's risk; the assessment must be modified appropriately based on the unique risks faced by each laboratory for the specific work being done. **The four groups address the risk to both the laboratory worker and the community and correlate with, but do not equate to, Biosafety Levels.** See Section III for additional information about Risk Groups and Biosafety Levels.

Genetically modified agent hazardous characteristics The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase or decrease an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk assessment can be difficult or incomplete because important information may not be available for a newly engineered agent. Several investigators have reported that they observed unanticipated enhanced virulence in recent studies with engineered agents;[7–10] these observations give reasons to remain alert to the possibility that experimental alteration of virulence genes may lead to altered risk and reinforce the nature of risk assessment as a continuing process that requires updating as research progresses.

The *NIH Guidelines* are the key reference in assessing risk and establishing an appropriate Biosafety Level for work involving recombinant DNA molecules. Please refer to Appendix J for more information about the *NIH Guidelines* and the NIH Office of Science Policy (OSP). The NIH Guidelines are available at https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf.[11]

Cell Cultures Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is illustrated by the reactivation of herpes viruses from latency,[12,13] the inadvertent transmission of disease to organ recipients,[14,15] and the persistence of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) within infected individuals in the U.S. population.[16] In addition, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce an infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple LAIs.[17] See Appendix H for additional information.

Other hazardous characteristics of an agent include probable routes of transmission in the laboratory, infective dose, stability in the environment, host range, and its endemic nature. In addition, reports of LAIs are a clear indicator of hazard and often are sources of information helpful for identifying agent and procedural hazards, and the precautions for their control. The absence of a report does not indicate minimal risk. The number of infections reported for a single agent may be an indication of the frequency of use as well as risk. Reporting of LAIs by laboratory directors in scientific and medical literature is encouraged. The agent summary statements in BMBL include specific references to reports on LAIs.

Once the inherent risk associated with the agent is considered, the next step in the process involves addressing the possibility of transmission of the agent. The most likely routes of transmission in the laboratory are:

1. Direct skin, eye or mucosal membrane exposure to an agent;
2. Parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors;
3. Ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and
4. Inhalation of infectious aerosols.

An awareness of the routes of transmission for the natural human disease is helpful in identifying probable routes of transmission in the laboratory and the potential for any risk to public health. For example, transmission of infectious agents can occur by direct contact with discharges from respiratory mucous membranes of infected persons, which would be a clear indication that a laboratory worker is at risk of infection from mucosal membrane exposure to droplets generated while handling that agent. Additional information used to identify both natural and often noted laboratory modes of transmission can be found in the *Control of Communicable Diseases Manual*.[3] It is important to remember that the nature and severity of disease caused by a Laboratory-associated infection and the probable route of transmission of the infectious agent in the laboratory may differ from the route of transmission and severity associated with the naturally-acquired disease.[18]

An agent capable of transmitting disease through respiratory exposure to infectious aerosols is a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. Infective dose and agent stability are particularly important in establishing the risk of airborne transmission of disease. For example, the reports of multiple infections in laboratories associated with the use of *Coxiella burnetii* are explained by its low inhalation infective dose, which is estimated to be 10 inhaled infectious particles, and its resistance to environmental stresses that enables the agent to survive outside of a living host or culture media long enough to become an aerosol hazard.[19]

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents require careful consideration when completing a risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an important indicator of hazard. The death of a primate center laboratory worker from Macacine herpesvirus 1 (MHV-1, also known as Monkey B virus) infection following an ocular splash exposure to biologic material from a rhesus macaque emphasizes the seriousness of this hazard.[20] Experiments that demonstrate

transmission of disease from an infected animal to a normal animal housed in the same cage are reliable indicators of hazard. Experiments that do not demonstrate transmission, however, do not rule out the hazard. For example, experimental animals infected with *Francisella tularensis*, *Coxiella burnetii*, *Coccidioides immitis*, or *Chlamydia psittaci*—agents that have caused many LAIs—rarely infect cagemates.[21]

The origin of the agent is also important when conducting a risk assessment. Non-indigenous agents are of special concern because of their potential to transmit or spread infectious diseases from foreign countries into the United States. Importation of agents of human disease requires a permit from the CDC. Importation of many agents of livestock, poultry, and other animal diseases requires a permit from the USDA's Animal and Plant Health Inspection Service (APHIS). For additional details, see Appendix C.

Often, there is not sufficient information to make an appropriate assessment of risk. For example, the hazard of an unknown agent that may be present in a specimen may not be known until the completion of agent identification and typing procedures. It would be prudent to assume the specimen contains an unknown agent presenting the hazardous classification that correlates with a minimum of BSL-2 containment, unless additional information suggests the presence of an agent of higher risk. Identification of agent hazards associated with newly emergent pathogens also requires judgments based on incomplete information. Often, epidemiologic findings are the best sources for information in these cases. When assessing the hazards of a newly attenuated pathogen, experimental data should support a judgment that the attenuated pathogen is less hazardous than the wild-type parent pathogen before making any reduction in the containment recommended for that pathogen.

Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

Investigations of LAIs have identified the following routes of transmission: parenteral inoculations with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, animal bites and scratches, and inhalation exposures to infectious aerosols. The first four routes of laboratory transmission were easy to detect but accounted for less than 20% of the LAIs reported in the 1979 retrospective review by Pike.[22] Subsequent research on LAIs has confirmed that the probable sources of infection are frequently not known.[23]

Aerosols and droplets Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of exposure. There is general agreement among biosafety professionals, laboratory directors, and principal investigators who have investigated LAIs that an aerosol generated by procedures and operations is the probable source of many LAIs, particularly in cases involving workers whose only known risk factor was that they worked with an agent or were in an area where that work was done.

Procedures that impart energy to a microbial suspension will produce aerosols. Equipment used for handling and analyzing infectious agents in laboratories, such as pipettes, blenders, centrifuges, sonicators, vortex mixers, cell sorters, and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometers are potential sources of aerosols.[24,25] These procedures and equipment generate respirable-size particles that remain airborne for protracted periods. These particles can remain in the lungs if inhaled or create an exposure hazard for coworkers in the laboratory or persons occupying adjacent spaces open to airflow from the laboratory. A number of investigators have determined the aerosol output of common laboratory procedures. In addition, investigators have proposed a model for estimating inhalation dosage from a laboratory aerosol source. Parameters that characterize aerosol hazards include an agent's inhalation infective dose, its viability in an aerosol, aerosol concentration, and particle size.[26–28]

A careful and proficient worker will minimize the generation of aerosols. For example, the hurried worker may operate a sonic homogenizer with maximum aeration, but the careful worker will consistently operate the device to ensure minimal aeration. Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration.[26] Similar results were shown for improper pipetting which generated bubbles versus pipetting with minimal bubble generation.

Procedures and equipment that generate respirable size particles also generate larger size droplets that settle out of the air rapidly, contaminating hands, work surfaces, and possibly the mucous membranes of the persons performing the procedure. An evaluation of the release of both respirable particles and droplets from laboratory operations determined that the respirable component is relatively small; in contrast, hand and surface contamination can be substantial.[29] The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Personal Protective Equipment (PPE) and Safety Equipment Hazards There may be hazards that require specialized PPE in addition to safety glasses,

laboratory gowns, and gloves. For example, a procedure that presents a splash hazard may require the use of a mask and a face shield to provide adequate protection. Inadequate training in the proper use of PPE may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator worn incorrectly may impart a risk to the wearer independent of the agents being manipulated.

Safety equipment such as biological safety cabinets (BSCs), centrifuge safety cups, and sealed rotors are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets. Safety equipment that is not working properly is hazardous, especially when the user is unaware of the malfunction. Poor location, room air currents, decreased airflow, leaking filters, raised sashes, crowded work surfaces, and poor user technique compromise the containment capability of a BSC. The safety characteristics of modern centrifuges are only effective if the equipment is operated properly.

Facility Control Hazards Facility safeguards help prevent the accidental release of an agent from the laboratory. For example, one facility safeguard is directional airflow, which helps to prevent aerosol transmission from a laboratory into other areas of the building. Directional airflow is dependent on the operational integrity of the laboratory's heating, ventilation, and air conditioning (HVAC) system. HVAC systems require careful monitoring and periodic maintenance to sustain operational integrity. Loss of directional airflow may compromise safe laboratory operation. BSL-4 containment facilities provide more complex safeguards that require significant expertise to design and operate.

Consideration of facility safeguards is an integral part of the risk assessment. A biological safety professional, building and facilities staff, and the IBC, or equivalent safety committee, should help assess the facility's capability to provide appropriate protection for the planned work and recommend changes as necessary. Risk assessment may support the need to include additional facility safeguards in the construction of new or renovation of old facilities.

Third, make a determination of the appropriate Biosafety Level and select additional precautions indicated by the risk assessment. The selection of the appropriate Biosafety Level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards described in Sections III, IV, and V of this publication.

There will be situations where the intended use of an agent requires greater precautions than those described in the agent's summary statement. These situations will require the careful selection of additional precautions. An obvious example would be a procedure for exposing animals to experimentally generated infectious aerosols.

It is unusual that a risk assessment would indicate a need to alter the recommended facility safeguards specified for the selected Biosafety Level. If this does occur, it is important that a biological safety professional validate this judgment before augmenting any facility secondary barrier.

While an entity's biosafety plan is based on a risk assessment, the biosafety plan may be influenced by federal regulations and guidelines. For example, the 2017 notice published by the National Science Foundation (NSF) defines standard terms and conditions for federal research grants.[30] A listing of statutory, regulatory, and executive requirements is provided in Appendix C of the updated National Policy Requirements Matrix.[31] The biosafety plan required by the Federal Select Agents and Toxins regulations (9 CFR Part 121, 42 CFR Part 73) must be based on an assessment that addresses the risk of the Select Agent or Toxin given its intended use and consider, where appropriate, the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Pre-existing conditions, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure of infants to certain agents are some of the conditions that may increase the risk of an individual for acquiring an LAI. Consultation with an occupational health care provider knowledgeable in infectious diseases is advisable in these circumstances.

Laboratory directors and principal investigators, or their designees, are responsible for ensuring that the identified controls (equipment, administrative, and PPE) have been made available and are adhered to or operating properly. For example, a BSC that is not certified represents a potentially serious hazard to the laboratory worker using it and to others in the laboratory. The director should have all equipment deficiencies corrected before starting work with an agent. Vaccination(s) may be recommended for laboratory personnel based on safety and availability; however, the protection afforded by a vaccine to an individual depends on the effectiveness of the vaccine and duration of immunity. Vaccination does not substitute for engineering and administrative risk mitigation controls.

Institutions must address risk perception by setting risk tolerance limits or performance expectations on program elements and equipment identified as critical to operations.[32,33] Risk mitigation requires finding a balanced approach that includes ongoing hazard identification and review of control measures with a commitment at all levels to reduce identified risk to a level tolerable to the institution. Risk acceptance is not equal acceptance of all risks; a level of biological risk may be essential to performing research, while acceptance of an equal risk of scientific misconduct is not.

Fourth, before implementation of the controls, review the risk assessment and selected safeguards with a biosafety professional, subject matter expert, and the IBC or equivalent resource. This review is strongly recommended and may be required by regulatory or funding agencies. Review of potentially high-risk protocols by the IBC should become standard practice. Adopting this step voluntarily will promote the use of safe practices in work with hazardous agents in microbiological and biomedical laboratories.

Fifth, as part of an ongoing process, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment. The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. The laboratory director or principal investigator should ensure that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent and have developed good habits that sustain excellence in the performance of those practices. Staff at all skill levels need to know how to identify hazards in the laboratory and how to obtain assistance in protecting themselves and others in the laboratory. An evaluation of a worker's training, experience in handling infectious agents, proficiency in following good microbiological practices, correct use of safety equipment, consistent use of standard operating procedures (SOPs) for specific laboratory activities, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is an important indication that a laboratory worker is capable of working safely.

An assessment should identify any potential deficiencies in the knowledge, competency, and practices of the laboratory workers. Carelessness is a serious concern because it can compromise any safeguards of the laboratory and increase the risk for coworkers. Fatigue and its adverse effects on safety have been well documented.[34] Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for laboratory staff in order to reduce the risks associated with work with hazardous agents. Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials. Laboratory directors or principal investigators should consider the use of competency assessment(s) to train and retrain new staff to the point where aseptic techniques and safety precautions become second nature.[35–37]

Sixth, revisit regularly and verify risk management strategies and determine if changes are necessary. Continue the risk management cycle, and adjust and adapt as the need arises. This includes a regular update of biosafety manuals and SOPs when changes in procedures or equipment occur. A cyclical, adaptable

risk management process forms the basis for a robust culture of safety in the biological laboratory.

Risk Communication

An effective culture of safety depends on the effective communication and reporting of risk indicators, including incidents and near misses, in a non-punitive manner.[38] Documents communicating the fundamental elements of a safety program are an important part of this culture and form the basis of the risk assessment; this includes hazard communication to all stakeholders.[39] Institutional leadership can engage workers at all levels by collaborating with institutional safety programs and committing to and supporting a safe working environment.

Institutions that work with infectious agents and toxins need an appropriate organizational and governance structure to ensure compliance with biosafety, biocontainment, and laboratory biosecurity regulations and guidelines, and to communicate risks.[40] In particular, the principal investigator or the facility equivalent has the primary responsibility for communicating hazards and risks in the laboratory. Staff must have the ability to report issues, including incidents and near misses without fear of reprisal. Laboratory staff, IBCs or equivalent resource, biosafety professionals, Institutional Animal Care and Use Committees (IACUCs), and laboratory animal veterinarians also have responsibility for identifying biological risks associated with laboratory work and communicating institute-wide risk management practices. A biosafety officer (BSO) and/or other safety personnel can coordinate the institution's safety program and may assist in the development of risk communication documents including incident trends and mitigations, SOPs, biosafety manuals, hazard control plans, and emergency response plans. Risk management can identify deficiencies in laboratory worker performance or institutional policies and assists institutional leadership responsible to make the necessary changes to safety programs to address those deficiencies. Biosafety program changes that promote the building of a culture of safety are most effectively communicated across the institution using multiple communication routes to ensure that all staff are informed. Good communication practices include messages from leadership, risk management documents, IBCs or equivalent resource, and other committee reviews, as necessary.

Facilitating a Culture of Safety through Risk Assessment

The goal of your risk assessment is to address all realistic, perceivable risks to protect personnel, the community, and the environment. Research progress, changes in personnel, and changes in regulation over time drive programmatic change and demand reconsideration of all factors, as periodically necessary. Risk assessment is an ongoing process, and all personnel have a role in its success.

The challenge is to develop good habits and procedures through training and competency checks with the support of leadership. Once established, these practices will persist to further instill a culture of safety. A sound risk communication strategy is also critical for both hazard identification and successful implementation. While policies and plans are tangible assets derived from the risk assessment process, the ultimate success will be measured by whether you establish, strengthen, and sustain a culture of safety while encouraging communication about risks between management and staff to prevent accidents before they happen.

The regular review of all hazards, prioritization of risk, multidisciplinary review of priority risks, and establishment of risk mitigation measures demonstrate the institution's commitment to a safe and secure working environment and form the cornerstone of a biosafety program. The approach to risk assessment outlined in the preceding section is not static and benefits from active participation by all relevant stakeholders. Aim for ongoing evaluation and periodic readjustments to stay aligned with the changing needs of the institution and to protect all persons from potential exposure to biological materials in laboratories and associated facilities.

Conclusion

The BMBL is designed to assist organizations with the protection of workers in biological laboratories and associated facilities from Laboratory-associated infections. Risk assessment is the basis for the safeguards developed by the CDC, the NIH, and the microbiological and biomedical community to protect the health of laboratory workers and the public from the risks associated with the use of hazardous biological agents in laboratories. Experience shows that these established safe practices, equipment, and facility safeguards work; new knowledge and experience may justify altering these safeguards.

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