Biosafety in Microbiological and Biomedical Laboratories

6th Edition

Centers for Disease Control and Prevention
National Institutes of Health

Inside front cover

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U.S. Department of Health and Human Services Public Health Service
Centers for Disease Control and Prevention
National Institutes of Health

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Foreword

Biosafety in Microbiological and Biomedical Laboratories (BMBL) has served as the cornerstone of biosafety practice in the United States since its initial release. We wish to emphasize that the sixth edition of BMBL remains an advisory document recommending best practices for the safe conduct of work in biomedical and clinical laboratories from a biosafety perspective. The BMBL is not intended to be a regulatory document although we recognize that some may use it in that way. The core principle of this document is protocol-driven risk assessment; it is not possible for a single document to identify all of the possible combinations of risks and mitigations feasible in biomedical and clinical laboratories. The BMBL should be used as a tool in the assessment and proposed mitigation steps in biomedical and clinical laboratories.

This edition of BMBL includes revised sections, agent summary statements, and appendices. We harmonized the recommendations included in this edition with guidance issued and regulations promulgated by other organizations and federal agencies. Wherever possible, we clarified both the language and intent of the information provided. In order to serve the needs of our community better, this edition includes new appendices on the following topics: inactivation and verification; laboratory sustainability; large-scale biosafety; and clinical laboratory biosafety.

Over 200 of our scientific and professional colleagues contributed to the preparation of the sixth edition through participation in technical working groups and serving as reviewers, guest editors, and subject matter experts. We wish to thank them all for their dedication and hard work. Without them, the sixth edition of BMBL would not be possible. We also recognize the hard work and contributions made by all who participated in preparation of the previous editions of BMBL; we have built on their solid work and commitment.

It would have been impossible to publish this revision without recognizing the visionary leadership of the previous BMBL editors—Drs. John Richardson, W. Emmett Barkley, Jonathan Richmond, Robert W. McKinney, Casey Chosewood, and Deborah Wilson—without whom the BMBL would not be the respected resource it is today. The Steering Committee members, Drs. Christy Myrick, Richard G. Baumann, Margy Lambert, Patricia Delarosa, and Theresa Lawrence, were instrumental in identifying authors, selecting additions to this edition, and reviewing submissions. Their significant contribution to this edition is sincerely appreciated.

We are truly grateful to Ms. Shaina Mangino and Dr. Mallory Pomales of Eagle Medical Services, LLC for their expertise and patience in assisting us with this

undertaking. Their superb organizational and editing skills were critical in the creation of this document.

We hope you find the sixth edition of *Biosafety in Microbiological and Biomedical Laboratories* complete, timely, and most of all, easy to use. Thank you for your patience and understanding during the long and comprehensive revision process.

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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.