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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

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Foreword

Biosafety in Microbiological and Biomedical Laboratories (BMBL) quickly became the cornerstone of biosafety practice and policy in the United States upon first publication in 1984. Historically, the information in this publication has been advisory in nature even though legislation and regulation, in some circumstances, have overtaken it and made compliance with the guidance provided mandatory. We wish to emphasize that the 5th edition of the BMBL remains an advisory document recommending best practices for the safe conduct of work in biomedical and clinical laboratories from a biosafety perspective, and is not intended as a regulatory document though we recognize that it will be used that way by some.

This edition of the BMBL includes additional sections, expanded sections on the principles and practices of biosafety and risk assessment; and revised agent summary statements and appendices. We worked to harmonize the recommendations included in this edition with guidance issued and regulations promulgated by other federal agencies. Wherever possible, we clarified both the language and intent of the information provided. The events of September 11, 2001, and the anthrax attacks in October of that year re-shaped and changed, forever, the way we manage and conduct work in biological and clinical laboratories and drew into focus the need for inclusion of additional information in the BMBL. To better serve the needs of our community in this new era, this edition includes information on the following topics:

- Occupational medicine and immunization
- Decontamination and sterilization
- Laboratory biosecurity and risk assessment
- Biosafety level 3 (Ag) laboratories
- Agent summary statements for some agricultural pathogens
- Biological toxins

At last count, over two hundred of our scientific and professional colleagues have assisted in the preparation of the 5th edition through participation in technical working groups, serving as reviewers and guest editors, and as subject matter experts. We wish to thank them all for their dedication and hard work for without them the 5th edition of the 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 the BMBL; we have built on their solid work and commitment. It is impossible to publish this revision without recognizing the visionary leadership of the previous BMBL editors, Drs. John Richardson and W. Emmett Barkley, and Drs. Jonathan Richmond and Robert W. McKinney, without whom the BMBL would not be the widely and well-regarded resource it is today. The Executive Steering Committee did a stellar job in shepherding this massive revision effort

and not without many bumps and bruises along the way. It is through their absolute commitment to quality, technical accuracy, and dedication to the professional practice of biosafety that the 5th edition is born. We are truly grateful to Ms. Kerstin Traum, Council Rock Consulting for her expertise, keen eye for detail and seemingly tireless efforts in performing the duties of technical writer-editor. We also gratefully acknowledge Ms. Cheryl Warfield of Proven Practices, LLC for her copy-editing and formatting skills that significantly enhanced this edition's readability and ease of use.

Finally, without the superb project management abilities and leadership of Dr. Joseph McDade and the technical/scientific editing expertise of Dr. Karl Johnson, especially in virology, the 5th edition of the BMBL would not be possible.

We hope you find this 5th 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. We believe you will find it was well worth the wait.

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Contributors

BMBL is by its nature a continuously revised manual, and each revision refines and extends the contributions to previous editions. Since 1984, when the first edition of BMBL was published, many scientists and biosafety specialists have contributed to this important reference work. The 5th edition is no exception, as specialists in multiple disciplines generously provided their considerable expertise to this revision. The Editors and Steering Committee gratefully acknowledge the contributions of all of these many contributors over the life of the BMBL, especially contributors to the current edition, who are listed below.

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Section I—Introduction

Over the past two decades, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) has become the code of practice for biosafety—the discipline 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 BMBL¹ and carried through in this fifth 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 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 code of practice will continue to serve the microbiological and biomedical community as a relevant and valuable authoritative reference.

We are living in an era of uncertainty and change. 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. Recent 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 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 twentieth 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 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 sub-clinical or asymptomatic infections.

In addition, 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 sub-clinical infections. Like Pike and Sulkin, Harding and Byers reported that only a small number of the LAI involved a 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 sub-clinical 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 with any degree of certainty.

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 biological research.

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 will reduce LAIs and protect the public health and 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 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 from man.^{12,13} In 1976 NIH first published the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*.¹⁴ The *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 emerging technology. 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. 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 fifth 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 a particular agent. The basic practices and equipment are appropriate for protocols common to most research and clinical laboratories. The facility safeguards help protect non-laboratory occupants of the building and the public health and environment.

Biosafety level 1 (BSL-1) is the basic level of protection and is appropriate for agents that are not known to cause disease in normal, healthy 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) standards.

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 the 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. Most manipulations of liquid suspensions of microorganisms 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 those 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 fifth 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 has 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 biosafety committees; biosafety officers; and public health, biosafety, and scientific associations are excellent resources.

The agent summary statements in the fourth edition BMBL were reviewed in the course of preparing the fifth edition of BMBL. There are new and updated agent summary statements including those for agents now classified as Select Agents. For example, there is an updated section on arboviruses and related zoonotic viruses including new agent summary statements. There are also substantive revisions to the Influenza Agent Summary Statement that address non-contemporary human influenza strains and recommend safeguards for research involving reverse genetics of the 1918 influenza strain.

The fifth edition also includes a revised section on risk assessment that gives more emphasis on the importance of this process in selecting the appropriate practices and level of containment. That section intentionally follows this introduction because risk assessment represents the foundation—a code of practice for safe handling of infectious agents in microbiological and biomedical laboratories.

Biosecurity

Today, the nation is facing a new challenge in safeguarding the public health from potential domestic or international terrorism involving the use of dangerous biological agents or toxins. Existing standards and practices may require adaptation to ensure protection from such hostile actions. In addition, recent federal regulations mandate increased security within the microbiological and biomedical community in order to protect biological pathogens and toxins from theft, loss, or misuse. The fifth edition of BMBL includes an important new section on 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 biosecurity concepts and guidelines introduced in this new section is essential for all laboratory workers.

Using BMBL

BMBL is both a code of practice and an authoritative reference. Knowledge sufficient to work safely with hazardous microorganisms requires a careful review of the entire 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 first edition of BMBL and expanded in the fifth edition are advisory in most circumstances. 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.

Looking Ahead

Laboratory-associated infections from exposure to biological agents known to cause disease are infrequent. It is critical that the microbiological and biomedical community continue its resolve to remain vigilant and not to become complacent. The LAIs reported in the last 25 years demonstrate that accidents and unrecognized exposures continue to occur. The absence of clear evidence of the means of transmission in most documented LAI 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 LAI¹⁵, 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.

The nation's response to recent world events brings with it a heightened concern for a potential increase in LAIs. In 2003, the United States federal government awarded significant funding for the construction of National Biocontainment Laboratories (NBL) and Regional Biocontainment Laboratories (RBL). The NBLs will house BSL-2, 3, and 4 laboratories; the RBLs will house BSL-2 and 3 laboratories. In addition, construction of new containment facilities by private and public institutions is underway nationwide. The expansion of biocontainment laboratories nationwide dramatically increases the need for training in microbiological practices and biosafety principles.

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

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Section II—Biological Risk Assessment

Risk assessment is an important responsibility for directors and principal investigators of microbiological and biomedical laboratories. Institutional biosafety committees (IBC), animal care and use committees, biological safety professionals, and laboratory animal veterinarians share in this responsibility. Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.

Laboratory directors and principal investigators should use risk assessment to alert their staffs to the hazards of working with infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building, and the public.

Risk assessment requires careful judgment. Adverse consequences are more likely to occur if the risks are underestimated. By contrast, imposition of safeguards more rigorous than actually needed may result in additional expense and burden for the laboratory, with little safety enhancement. Unnecessary burden may result in circumvention of required safeguards. However, where there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data are available.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. In addition, the capability of the laboratory staff to control hazards must be considered. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

The agent summary statements contained in BMBL identify the primary agent and procedure hazards for specific pathogens and recommend precautions for their control. The guest editors and contributors of this and previous editions of BMBL based their recommendations on an assessment of the risks associated with the handling of pathogens using generally routine generic laboratory procedures. A review of the summary statement for a specific pathogen is a helpful starting point for assessment of the risks of working with that agent and those for a similar agent.

Hazardous Characteristics of an Agent

The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease. The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on these principal characteristics and the route of transmission of the natural disease.¹ The four groups address the risk to both the laboratory worker and the community. The *NIH Guidelines* established a comparable classification and assigned human etiological agents into four risk groups on the basis of hazard.² The descriptions of the WHO and NIH risk group classifications are presented in Table 1. They correlate with but do not equate to biosafety levels. A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level. See Section 3 for a further discussion of the differences and relatedness of risk groups and biosafety levels.

Table 1: Classification of Infectious Microorganisms by Risk Group

Risk Group Classification	NIH Guidelines for Research involving Recombinant DNA Molecules 2002 ²	World Health Organization Laboratory Biosafety Manual 3 rd Edition 2004 ¹
Risk Group 1	Agents not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism unlikely to cause human or animal disease.
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. ³

Other hazardous characteristics of an agent include probable routes of transmission of laboratory infection, 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. Reports seldom provide incidence data, making comparative judgments on risks among agents difficult. The number of infections reported for a single agent may be an indication of the frequency of use as well as risk. Nevertheless, reporting of LAIs by laboratory directors in the scientific and medical literature is encouraged. Reviews of such reports and analyses of LAIs identified through extensive surveys are a valuable resource for risk assessment and reinforcement of the biosafety principles. The summary statements in BMBL include specific references to reports on LAIs.

The predominant probable 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 the 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. The American Public Health Association publication *Control of Communicable Diseases Manual* is an excellent reference for identifying both natural and often noted laboratory modes of transmission.³ However, it is important to remember that the nature and severity of disease caused by a laboratory infection and the probable laboratory route of transmission of the infectious agent may differ from the route of transmission and severity associated with the naturally-acquired disease.⁴

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. This hazard requires special caution because infectious aerosols may not be a recognized route of transmission for the natural disease. 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 ten 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.⁵

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents require careful consideration in 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 Cercopithecine herpes virus 1 (CHV-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.⁶ Lack of awareness for this potential hazard can make laboratory staff vulnerable to an unexpected outbreak involving multiple infections.⁷ 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 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.⁸

The origin of the agent is also important in risk assessment. Non-indigenous agents are of special concern because of their potential to introduce risk of transmission, or spread of human and animal or infectious diseases from foreign countries into the United States. Importation of etiological agents of human disease requires a permit from the CDC. Importation of many etiological 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.

Genetically modified agent hazards. 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 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.⁹⁻¹² These observations give reason to remain alert to the possibility that experimental alteration of virulence genes may lead to increased risk. It also suggests that risk assessment is 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.² The purpose of the *NIH Guidelines* is to promote the safe conduct of research involving recombinant DNA. The guidelines specify appropriate practices and procedures for research involving constructing and handling both recombinant DNA molecules and organisms and viruses that contain recombinant DNA. They define recombinant DNA as a molecule constructed outside of a living cell with the capability to replicate in a living cell. The *NIH Guidelines* explicitly address experiments that involve introduction of recombinant DNA into Risk Groups 2, 3,

and 4 agents, and experiments in which the DNA from Risk Groups 2, 3, and 4 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. Compliance with the *NIH Guidelines* is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for recombinant DNA research. Many other institutions have adopted these guidelines as the best current practice.

The *NIH Guidelines* were first published in 1976 and are revised on an ongoing basis in response to scientific and policy developments. The guidelines outline the roles and responsibilities of various entities affiliated with recombinant DNA research, including institutions, investigators, and the NIH. Recombinant DNA research subject to the *NIH Guidelines* may require: 1) approval by the NIH Director, review by the NIH Recombinant DNA Advisory Committee (RAC), and approval by the IBC; or 2) review by the NIH Office of Biotechnology Activities (OBA) and approval by the IBC; or 3) review by the RAC and approvals by the IBC and Institutional Review Board; or 4) approval by the IBC prior to initiation of the research; or 5) notification of the IBC simultaneous with initiation of the work. It is important to note that review by an IBC is required for all non-exempt experiments as defined by the *NIH Guidelines*.

The *NIH Guidelines* were the first documents to formulate the concept of an IBC as the responsible entity for biosafety issues stemming from recombinant DNA research. The *NIH Guidelines* outline the membership, procedures, and functions of an IBC. The institution is ultimately responsible for the effectiveness of the IBC, and may define additional roles and responsibilities for the IBC apart from those specified in the *NIH Guidelines*. See Appendix J for more information about the *NIH Guidelines* and OBA.

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 well understood and illustrated by the reactivation of herpes viruses from latency,^{13,14} the inadvertent transmission of disease to organ recipients,^{15,16} and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population.¹⁷ There also is evidence of accidental transplantation of human tumor cells to healthy recipients which indicates that these cells are potentially hazardous to laboratory workers who handle them.¹⁸ 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.¹⁹ The potential for human cell lines to harbor a bloodborne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational exposure to bloodborne pathogens final rule would include primary human cell lines and explants.¹⁷

Hazardous Characteristics of Laboratory Procedures

Investigations of LAIs have identified five principal routes of laboratory transmission. These are 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 are easy to detect, but account for less than 20 percent of all reported LAIs.²⁰ Most reports of such infections do not include information sufficient to identify the route of transmission of infection. Work has shown that the probable sources of infection—animal or ectoparasite, clinical specimen, agent, and aerosol—are apparent in approximately 50 percent of cases.²¹

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 infection. 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 in an area where that work was done.

Procedures that impart energy to a microbial suspension will produce aerosols. Procedures and equipment used routinely for handling infectious agents in laboratories, such as pipetting, blenders, non-self contained centrifuges, sonicators and vortex mixers are proven sources of aerosols. These procedures and equipment generate respirable-size particles that remain airborne for protracted periods. When inhaled, these particles are retained in the lungs creating an exposure hazard for the person performing the operation, coworkers in the laboratory, and a potential hazard for persons occupying adjacent spaces open to air flow 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.^{22,23,24}

Procedures and equipment that generate respirable size particles also generate larger size droplets that can contain multiple copies of an infectious agent. The larger size droplets settle out of the air rapidly, contaminating the gloved hands and work surface 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 and does not vary widely; in contrast hand and surface contamination is substantial and varies widely.²⁵ The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Technique can significantly impact aerosol output and dose. The worker who is careful and proficient will minimize the generation of aerosols. A careless and hurried worker will substantially increase the aerosol hazard. For example, the hurried worker may operate a sonic homogenizer with maximum aeration whereas the careful worker will consistently operate the device to assure minimal aeration. Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration.²² Similar results were shown for pipetting with bubbles and with minimal bubbles. Containment and good laboratory practices also reduce this risk.

Potential Hazards Associated with Work Practices, Safety Equipment and Facility Safeguards

Workers are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents. Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment. A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Carelessness is the most serious concern, because it can compromise any safeguards of the laboratory and increase the risk for coworkers. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for a laboratory staff in order to reduce the inherent risks that attend 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 train and retrain new staff to the point where aseptic techniques and safety precautions become second nature.²⁶

There may be hazards that require specialized personal protective equipment 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 personal protective equipment may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator may impart a risk to the wearer independent of the agents being manipulated.

Safety equipment such as biological safety cabinets (BSC), 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. Training in the correct use of equipment, proper procedure, routine inspections

and potential malfunctions, and periodic re-certification of equipment, as needed, is essential.

Facility safeguards help prevent the accidental release of an agent from the laboratory. Their use is particularly important at BSL-3 and BSL-4 because the agents assigned to those levels can transmit disease by the inhalation route or can cause life-threatening disease. For example, one facility safeguard is directional airflow. This safeguard 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 compromises 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 assessments. A biological safety professional, building and facilities staff, and the IBC 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 BSL-3 facilities.

An Approach to Assess Risks and Select Appropriate Safeguards

Biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures, with judgments based often on incomplete information. There is no standard approach for conducting a biological risk assessment, but some structure can be helpful in guiding the process. This section describes a five-step approach that gives structure to the risk assessment process.

First, identify agent hazards and perform an initial assessment of risk.

Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.

Several excellent resources provide information and guidance for making an initial risk assessment. The BMBL provides agent summary statements for some 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 infection and, when available, information on infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent.

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 summary statement or when an agent summary statement is

not available. Although a summary statement for one agent may provide helpful information for assessing the risk of a similar agent, it should not serve as the primary resource for making the risk determination for that agent. Refer to other resources for guidance in identifying the agent hazards.

The *Control of Communicable Diseases Manual* provides information on communicable diseases including concise summaries on severity, mode of transmission, and the susceptibility and resistance of humans to disease.³ In addition, it is always helpful to seek guidance from colleagues with experience in handling the agent and from biological safety professionals.

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 diagnostic specimen will be unknown until after completing agent identification and typing procedures. It would be prudent in this case to assume the specimen contains an agent presenting the hazardous classification that correlates with BSL-2, 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. Consult interim biosafety guidelines prepared by the CDC and the WHO for risk assessment guidance. 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.

Make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. Remember that aerosol and droplet routes of agent transmission also are important considerations in specification of safety equipment and facility design that result in a given BSL level.

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.

The complexity of a laboratory procedure can also present a hazard. The agent summary statement provides information on the primary laboratory hazards associated with typically routine procedures used in handling an agent. In proposed laboratory procedures where the procedure hazards differ from the general conditions of the agent summary statement or where an agent summary statement is not available, the risk assessment should identify specific hazards associated with the procedures.

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 unlikely that a risk assessment would indicate a need to alter the recommended facility safeguards specified for the selected biosafety level. If this does occur, however, it is important that a biological safety professional validate this judgment independently before augmenting any facility secondary barrier.

It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Pre-existing diseases, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure to infants to certain agents, are some of the conditions that may increase the risk of an individual for acquiring a LAI. Consultation with an occupational physician knowledgeable in infectious diseases is advisable in these circumstances.

Fourth, 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. In conducting a risk assessment, 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. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely.

The laboratory director or principal investigator should also ensure that the necessary safety equipment is available and 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.

Fifth, review the risk assessment with a biosafety professional, subject matter expert, and the IBC. A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the *NIH Guidelines*.² Review of potentially high risk protocols by the local 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.

Conclusion

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. Risk assessment, however, must be the basis for recommended change. Assessments conducted by laboratory directors and principal investigators for the use of emergent agents and the conduct of novel experiments will contribute to our understanding of the risks these endeavors may present and the means for their control. Those risk assessments will likely mirror progress in science and technology and serve as the basis for future revisions of BMBL.

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Section III—Principles of Biosafety

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term “containment” is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The use of vaccines may provide an increased level of personal protection. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Laboratory Practices and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The director or person in charge of the laboratory is responsible for providing or arranging the appropriate training of personnel.

Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures. A scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or materials. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment.

When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure.

Appropriate facility design and engineering features, safety equipment, and management practices must supplement laboratory personnel, safety practices, and techniques.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

Safety equipment includes BSCs, enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of

infectious droplets or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II BSCs are primary barriers that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize aerosol hazards, containment controls such as BSCs or centrifuge cups must be used when handling infectious agents.

Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with BSCs and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in BSCs, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

Facility Design and Construction (Secondary Barriers)

The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Laboratory directors are responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to

ensure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules to isolate the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the ASHRAE Laboratory Design Guide published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).¹

Biosafety Levels

Four BSLs are described in Section 4, which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity. The BSLs described in this manual should be differentiated from Risk Groups, as described in the *NIH Guidelines* and the World Health Organization Laboratory Biosafety Manual. Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

The recommended biosafety level(s) for the organisms in Section VIII (Agent Summary Statements) represent those conditions under which the agent ordinarily can be safely handled. Of course, not all of the organisms capable of causing disease are included in Section VIII and an institution must be prepared to perform risk assessments for these agents using the best available information. Detailed information regarding the conduct of biological risk assessments can be found in Section II. The laboratory director is specifically and primarily responsible for assessing the risks and applying the appropriate biosafety levels. The institution's Biological Safety Officer (BSO) and IBC can be of great assistance in performing and reviewing the required risk assessment. At one point, under the *NIH Guidelines*, BSOs were required only when large-scale research or production of organisms containing recombinant DNA molecules was performed or when work with recombinant DNA molecules was conducted at BSL-3 or above. IBCs were required only when an institution was performing non-exempt recombinant DNA experiments. Today, however, it is strongly suggested that an institution conducting research or otherwise working with pathogenic agents have a BSO and properly constituted and functioning IBC. The responsibilities of each now extend beyond those described in the *NIH Guidelines* and depend on the size and complexity of the program.

Generally, work with known agents should be conducted at the biosafety level recommended in Section VIII. When information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent

practices may be specified. Often an increased volume or a high concentration of agent may require additional containment practices.

Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis*, *Nigeria gruberi*, infectious canine hepatitis virus, and exempt organisms under the *NIH Guidelines* are representative of microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains.

BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the *Salmonella*, and *Toxoplasma* are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard² for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers, such as hand washing sinks and waste decontamination facilities, must be available to reduce potential environmental contamination.

Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL-4.

The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

The laboratory director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel, procedures being conducted and the nature or function of the laboratory may further influence the director in applying these recommendations.

Animal Facilities

Four standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment.

One additional biosafety level, designated BSL-3-Agriculture (or BSL 3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL 3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL 3-Ag facilities and USDA High Consequence Pathogens is provided in Appendix D.

Clinical Laboratories

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for “routine,” acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at BSL-2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL-2 are consistent with the OSHA standard, “Occupational Exposure to Bloodborne Pathogens.”^{2,3} This requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal or Standard* Precautions).^{4,5} Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute (formerly known as the National Committee for Clinical Laboratory Standards).⁶

BSL-2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as BSCs (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC (Class II) is indicated to protect the integrity of the specimen.

The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

Importation and Interstate Shipment of Certain Biomedical Materials

The importation of etiologic agents and vectors of human diseases is subject to the requirements of the Public Health Service Foreign Quarantine regulations. Companion regulations of the Public Health Service and the Department of Transportation specify packaging, labeling, and shipping requirements for etiologic agents and diagnostic specimens shipped in interstate commerce. (See Appendix C.)

The USDA regulates the importation and interstate shipment of animal pathogens and prohibits the importation, possession, or use of certain exotic animal disease agents that pose a serious disease threat to domestic livestock and poultry. (See Appendix F.)

Select Agents

In recent years, with the passing of federal legislation regulating the possession, use, and transfer of agents with high adverse public health and/or agricultural consequences (DHHS and USDA Select Agents), much greater emphasis has been placed in the emerging field of biosecurity. Biosecurity and select agent issues are covered in detail in Section 6 and Appendix F of this document. In contrast with biosafety, a field dedicated to the protection of workers and the environment from exposures to infectious materials, the field of biosecurity prevents loss of valuable research materials and limits access to infectious materials by individuals who would use them for harmful purposes. Nevertheless, adequate containment of biological materials is a fundamental program component for both biosafety and biosecurity.

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- * In 1996 the United States Hospital Infection Control Practices Advisory Committee introduced a new set of guidelines, "Standard Precautions," to synthesize the major features of Universal Precautions (blood and body fluid) with Body Substance Isolation Precautions (designed to reduce the risk of transmission of pathogens from moist body substances).⁶ Standard Precautions apply to 1) blood; 2) all body fluids, secretions, and excretions except sweat, regardless of whether or not they contain visible blood; 3) non-intact skin; and 4) mucous membranes. For additional information on Standard Precautions, see reference 6 or the CDC Web site: www.cdc.gov.

Section IV—Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 2 of this section and discussed in Section 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
 10. An effective integrated pest management program is required.
(See Appendix G.)

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

None required.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratories windows that open to the exterior should be fitted with screens.

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
 - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
 - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the

supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. (See Appendix G.)
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

- a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
 9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
 10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

C. Safety Equipment (*Primary Barriers and Personal Protective Equipment*)

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or

decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

- a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
 10. An effective integrated pest management program is required. (See Appendix G.)
 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. *Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.

3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

- a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
- b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
- c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
 - a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

- b. The laboratory exhaust air must not re-circulate to any other area of the building.
- c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
12. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BSL-4 laboratories:

1. A *Cabinet Laboratory*—Manipulation of agents must be performed in a Class III BSC; and
2. A *Suit Laboratory*—Personnel must wear a positive pressure supplied air protective suit.

BSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

The following standard and special safety practices, equipment, and facilities apply to BSL-4.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
3. Mechanical pipetting devices must be used.
4. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

Precautions, including those listed below, must be taken with any sharp items. These include:

- a. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - b. Use of needles and syringes or other sharp instruments should be restricted in the laboratory, except when there is no practical alternative.
 - c. Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal or decontamination. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal, located as close to the point of use as possible.
 - d. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
5. Perform all procedures to minimize the creation of splashes and/or aerosols.
 6. Decontaminate work surfaces with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.
 7. Decontaminate all wastes before removal from the laboratory by an effective and validated method.
 8. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
 9. An effective integrated pest management program is required. (See Appendix G.)
 10. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information

regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements in accordance with institutional policies.

Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes are authorized to enter.

Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the laboratory must be maintained.

While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All persons entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves (as appropriate). All persons leaving the laboratory must take a personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering.

After the laboratory has been completely decontaminated and all infectious agents are secured, necessary staff may enter and exit without following the clothing change and shower requirements described above.

2. Laboratory personnel and support staff must be provided appropriate occupational medical services including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired infections.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared. The biosafety manual must be available, accessible, and followed.
5. The laboratory supervisor is responsible for ensuring that laboratory personnel:
 - a. Demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL-4 containment.
 - b. Receive appropriate training in the practices and operations specific to the laboratory facility.
 - c. Receive annual updates and additional training when procedural or policy changes occur.
6. Removal of biological materials that are to remain in a viable or intact state from the laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside BSL-4 containment unless inactivated by a validated method.
7. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory.
 - b. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the laboratory. The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.
 - c. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the laboratory supervisor, institutional management and appropriate

laboratory personnel as defined in the laboratory biosafety manual. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. Supplies and materials that are not brought into the BSL-4 laboratory through the change room, must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility. The doors of the autoclave or fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave or fumigation chamber has been operated through a decontamination cycle.

Only necessary equipment and supplies should be stored inside the BSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the laboratory.

11. Daily inspections of essential containment and life support systems must be completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.
12. Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel and other responsible staff according to institutional policies.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Cabinet Laboratory

1. All manipulations of infectious materials within the laboratory must be conducted in the Class III biological safety cabinet.

Double-door, pass through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s). The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.

The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves. Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves.

Class III cabinet gloves must be inspected for damage prior to use and changed if necessary. Gloves should be replaced annually during cabinet re-certification.

The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible.

Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups.

The Class III cabinet must be certified at least annually.

2. Workers in the laboratory must wear protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. All protective clothing must be removed in the dirty side change room before showering. Reusable clothing must be autoclaved prior to removal from the laboratory for laundering.
3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eyeglasses must be decontaminated before removal through the personal body shower.

4. Disposable gloves must be worn underneath cabinet gloves to protect the worker from exposure should a break or tear occur in a cabinet glove. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

Suit Laboratory

1. All procedures must be conducted by personnel wearing a one-piece positive pressure supplied air suit.

All manipulations of infectious agents must be performed within a BSC or other primary barrier system.

Equipment that may produce aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as needed.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's specifications.

2. Workers must wear laboratory clothing, such as scrub suits, before entering the room used for donning positive pressure suits. All laboratory clothing must be removed in the dirty side change room before entering the personal shower.
3. Inner disposable gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to entering the personal shower. Dispose of used gloves with other contaminated waste.
4. Decontamination of outer suit gloves is performed during laboratory operations to remove gross contamination and minimize further contamination of the laboratory.

D. Laboratory Facilities (Secondary Barriers)

Cabinet Laboratory

1. The BSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the facility must be arranged to ensure sequential passage through an inner (dirty) changing area, a personal shower and an outer (clean) change room upon exiting the room(s) containing the Class III BSC(s).

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on an uninterrupted power supply (UPS).

A double-door autoclave, dunk tank, fumigation chamber, or ventilated airlock must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. A hands-free sink must be provided near the door of the cabinet room(s) and the inner change room. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC must be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory and inner change room must be sealed.

Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

Drains in the laboratory floor (if present) must be connected directly to the liquid waste decontamination system.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.

Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.
6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II cabinets should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.
8. An eyewash station must be readily available in the laboratory.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to and exhaust air from the cabinet room, inner change room, and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes.

All HEPA filters should be located as near as practicable to the cabinet and laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings should be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. If BSC exhaust is to be recirculated to the outside, BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a hard ducted, direct connection ensuring that cabinet exhaust air passes through two (2) HEPA filters—including the HEPA in the BSC—prior to release outside. Provisions to assure proper safety cabinet performance and air system operation must be verified.

Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable and airtight and capable of

expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented.

Suit Laboratory

1. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the facility must be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

Entry into the BSL-4 laboratory must be through an airlock fitted with airtight doors. Personnel who enter this area must wear a positive pressure suit supplied with HEPA filtered breathing air. The breathing air systems must have redundant compressors, failure alarms and emergency backup.

A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In the event of an emergency exit or failure of the chemical shower system, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

An automatically activated emergency power source must be provided, at a minimum, for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets.

Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on a UPS.

A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory.

2. Sinks inside the suit laboratory should be placed near procedure areas and be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed.

Drains, if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents must have protection against insect and animal intrusion.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Sharp edges and corners should be avoided. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.
6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the BSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.
9. A dedicated, non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to the laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.

All HEPA filters must be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings must be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable, airtight, and capable of expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented.

Table 2. Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	<ul style="list-style-type: none"> Not known to consistently cause diseases in healthy adults 	<p>Standard microbiological practices</p>	<ul style="list-style-type: none"> No primary barriers required. PPE: laboratory coats and gloves; eye, face protection, as needed 	<p>Laboratory bench and sink required</p>
2	<ul style="list-style-type: none"> Agents associated with human disease Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure 	<p>BSL-1 practice plus:</p> <ul style="list-style-type: none"> Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies 	<p>Primary barriers:</p> <ul style="list-style-type: none"> BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPE: Laboratory coats, gloves, face and eye protection, as needed 	<p>BSL-1 plus:</p> <ul style="list-style-type: none"> Autoclave available
3	<p>Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure</p>	<p>BSL-2 practice plus:</p> <ul style="list-style-type: none"> Controlled access Decontamination of all waste Decontamination of laboratory clothing before laundering 	<p>Primary barriers:</p> <ul style="list-style-type: none"> BSCs or other physical containment devices used for all open manipulations of agents PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed 	<p>BSL-2 plus:</p> <ul style="list-style-type: none"> Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory Entry through airlock or anteroom Hand washing sink near laboratory exit
4	<ul style="list-style-type: none"> Dangerous/exotic agents which pose high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level Related agents with unknown risk of transmission 	<p>BSL-3 practices plus:</p> <ul style="list-style-type: none"> Clothing change before entering Shower on exit All material decontaminated on exit from facility 	<p>Primary barriers:</p> <ul style="list-style-type: none"> All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit 	<p>BSL-3 plus:</p> <ul style="list-style-type: none"> Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decontamination systems Other requirements outlined in the text

Section V—Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for the laboratory animal. Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol-driven risk assessment.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., *Guide for the Care and Use of Laboratory Animals*¹ and *Laboratory Animal Welfare Regulations*²) and that appropriate species have been selected for animal experiments. In addition, the organization must have an occupational health and safety program that addresses potential hazards associated with the conduct of laboratory animal research. The following publication by the Institute for Laboratory Animal Research (ILAR), *Occupational Health and Safety in the Care and Use of Research Animals*³, is most helpful in this regard. Additional safety guidance on working with non-human primates is available in the ILAR publication, *Occupational Health and Safety in the Care and Use of Nonhuman Primates*.⁴

Facilities for laboratory animals used in studies of infectious or non-infectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities providing patient care. Traffic flow that will minimize the risk of cross contamination should be incorporated into the facility design.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These four combinations, designated Animal Biosafety Levels (ABSL) 1-4, provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices

applicable to work with animals infected with agents assigned to Biosafety Levels 1-4, respectively. Investigators that are inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

In addition to the animal biosafety levels described in this section, the USDA has developed facility parameters and work practices for handling agents of agriculture significance. Appendix D includes a discussion on Animal Biosafety Level 3 Agriculture (BSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. Appendix D also describes some of the enhancements beyond BSL/ABSL-3 that may be required by USDA-APHIS when working in the laboratory or vivarium with certain veterinary agents of concern.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in this section. The reader is referred to Appendix E for more information on the Arthropod Containment Guidelines.

Animal Biosafety Level 1

Animal Biosafety Level 1 is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. (See Section 2, Biological Risk Assessment.)

Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following standard practices, safety equipment, and facility requirements apply to ABSL-1.

A. *Standard Microbiological Practices*

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC)⁵ and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security-sensitive agent information should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.

Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designed and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative

for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

- b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Equipment containing sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
 13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.
 14. An effective integrated pest management program is required.
(See Appendix G.)
 15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

None required.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

2. Special containment devices or equipment may not be required as determined by appropriate risk assessment.
3. Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing.

Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.

4. Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with NHPs must assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields, etc.) as appropriate for the task to be performed.

5. Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

6. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. The animal facility must have a sink for hand washing.

Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

It is recommended that penetrations in floors, walls and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.¹ No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Animal Biosafety Level 2

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

A. *Standard Microbiological Practices*

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

Each organization must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study, animal protocols must also be reviewed and approved by the IACUC⁵ and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.

Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. The supervisor must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/ or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:

- a. The use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used, disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Use of equipment with sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
 13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or manipulated.
 14. An effective integrated pest management program is required.
(See Appendix G.)
 15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

When appropriate, a base line serum sample should be stored.

2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.

3. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.

A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented.

Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

4. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.

Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
6. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.

Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with NHPs should assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed. Respiratory protection is worn based upon risk assessment.

4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility.

If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.

Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.

Floors must be slip-resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.¹ The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.

All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.

12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
13. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.
14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Animal Biosafety Level 3

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

The ABSL-3 laboratory has special engineering and design features.

ABSL-3 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3.

A. Standard Microbiological Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study, animal protocols must be reviewed and approved by the IACUC⁵ and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures.

Consideration must be given to specific biohazards unique to the animal species and protocol in use.

3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with the research, animal husbandry duties, animal care, and manipulations.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having

these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

6. Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated.

All persons, including facility personnel, service workers, and visitors, are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious/hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:

- a. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used, disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Use of equipment with sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
 13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.
 14. An effective integrated pest management program is required.
(See Appendix G.)

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

When appropriate, a base line serum sample should be stored.

2. All procedures involving the manipulation of infectious materials, handling of infected animals or the generation of aerosols must be conducted within BSCs or other physical containment devices when practical.

When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

Restraint devices and practices are used to reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications).

3. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems.
4. Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate operational malfunctions.
5. A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials

and/or animals are housed or are manipulated (e.g., autoclave, chemical disinfection, or other approved decontamination methods).

Consideration must be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment.

Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) by an appropriate method before removal from the areas where infectious materials and/or animals are housed or manipulated.

It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or are manipulated, preferably within the caging system.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements.

6. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.

Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs and other physical containment devices or equipment should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

The risk of infectious aerosols from infected animals or bedding can be reduced by primary barrier systems. These systems may include solid

wall and bottom cages covered with filter bonnets, ventilated cage rack systems, or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

Personnel within the animal facility where protective clothing, such as uniforms or scrub suits. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.

Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility.

Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate eye, face and respiratory protection. To prevent cross contamination, boots, shoe covers, or other protective footwear, are used where indicated.

Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

4. Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Procedures may require the use of wearing two pairs of gloves (double-glove).

Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open.

Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.

2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated.

If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.

Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts and doorframes, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface.

Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases.

Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Equipment and furnishings with sharp edges and corners should be avoided.

5. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation of the facility should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*.¹ The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from “clean” areas and toward “contaminated” areas.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. HEPA filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes.

Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure

conditions the airflow will not be reversed. Alarms should be considered to notify personnel of ventilation and HVAC system failure.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or exhausted directly to the outside through a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance.

Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.

All BSCs should be used according to manufacturers' specifications.

When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

12. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate

infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials to designated alternate location/s within the facility.

13. Emergency eyewash and shower are readily available; location is determined by risk assessment.
14. The ABSL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.
15. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.

Animal Biosafety Level 4

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments; or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring ABSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or to re-designate the level. Animal care staff must have specific and thorough training in handling extremely hazardous, infectious agents and infected animals. Animal care staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All animal care staff and supervisors must be competent in handling animals, agents and procedures requiring ABSL-4 containment. The animal facility director and/or laboratory supervisor control access to the animal facility within the ABSL-4 laboratory in accordance with institutional policies.

There are two models for ABSL-4 laboratories:

1. A *Cabinet Laboratory*—All handling of agents, infected animals and housing of infected animals must be performed in Class III BSCs (see Appendix A); and
2. A *Suit Laboratory*—Personnel must wear a positive pressure protective suit (see Appendix A); infected animals must be housed in ventilated enclosures with inward directional airflow and HEPA filtered exhaust;

and infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. However, ABSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment and personnel. The ABSL-4 cabinet laboratory is distinctly different from an ABSL-3 laboratory containing a Class III BSC. The following standard and special safety practices, equipment, and facilities apply to ABSL-4.

A. Standard Microbiological Practices

1. The animal facility directors must establish and enforce policies, procedures, and protocols for biosafety, biosecurity and emergencies within the ABSL-4 laboratory.

The animal facility director and/or designated institutional officials are responsible for enforcing the policies that control access to the ABSL-4 facility. Laboratory personnel and support staff must be provided appropriate occupational medical service including medical surveillance and available immunizations for agents handled or potentially present in the laboratory.³ A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired infections. Facility supervisors should ensure that medical staff are informed of potential occupational hazards within the animal facility including those associated with the research, animal husbandry duties, animal care, and manipulations.

An ABSL-4 laboratory specific, biosafety manual must be prepared in consultation with the animal facility director, the laboratory supervisor, and the biosafety advisor. The biosafety manual must be available and accessible. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.

Prior to beginning a study, appropriate policies and procedures for animal welfare during the conduct of research, must be developed and approved by the IACUC. The biosafety official, the IBC and/or other applicable committees, are responsible for review of protocols and policies to prevent hazardous exposures to personnel who manipulate and care for animals.

2. A complete clothing change is required in the ABSL-4 operation. Personnel within the animal facility where protective clothing, such as uniforms or scrub suits.