Biosafety in Microbiological and Biomedical Laboratories

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and

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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.

During the latest revision of the BMBL additional chapters were added; chapters on the principles and practices of biosafety and on risk assessment were expanded; all agent summary statements and appendices were revised; and effort was made to harmonize recommendations with guidance issued and regulations promulgated by other federal agencies. Wherever possible, an attempt was made to clarify 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 draw into focus the need for inclusion of additional information in the BMBL. In an attempt to better serve the needs of our community in this new era, information on the following topics has been added in the 5th edition:

- 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 on 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 want to 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 seeming tireless efforts in performing the duties of technical writer-editor.

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 *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition complete, timely and most of all easy to use. Thank you for your patience and understanding during the long and comprehensive revision process. We know 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 BMBL[1] 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 sp., 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 was 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 sp., Salmonella sp., Shigella sp.*, hepatitis C virus, and *Cryptosporidium sp*. 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 breech 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 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. BMBL further expanded the technical content of the 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 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;

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 chapter on risk assessment that gives more emphasis on the importance of this process in selecting the appropriate practices and level of containment. That chapter 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 chapter 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 chapter 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 LAI[15], 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 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 PRACTICE 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.

Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

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 splashes 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 air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as 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 Chapter 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, to be 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 Chapter 8 (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 Chapter 8 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 Chapter 2. 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 in time, 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 to 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 Chapter 8. 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, Naegleria 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 handwashing.

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 salmonellae, and *Toxoplasma* spp. 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 Standard* [2] 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

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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 website (www.cdc.gov).

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 which 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 Chapter 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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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 1 of this chapter and discussed in Chapter 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. Plasticware 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 child-bearing 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, integrity has been 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. Plasticware 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 child-bearing 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 must establish policies and procedures describing the 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 safety 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 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 (preferably Class II), 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, 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, integrity has been 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 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 High Efficiency Particilate Air (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.

9. There are no specific requirements on 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 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 a direct (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 inhalation route 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, other physical containment devices, or by personnel wearing appropriate personal protective equipment.

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. Plasticware 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 child-bearing 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 must establish policies and procedures describing the 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 safety 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. Protective laboratory clothing with a solid-front such as tie-back or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.

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. 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 should:

    a. Change gloves when contaminated, integrity has been 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.

Access to the laboratory is restricted to entry by a series of 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 of the biological agents in use.

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 air flow. A visual monitoring device which confirms directional air flow 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.

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 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 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, incineration, or other validated decontamination method).

12. 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 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. 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.

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 life-threatening disease, aerosol transmission, or 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. Access to the laboratory is controlled by the laboratory supervisor in accordance with institutional policies.

There are two models for BSL-4 laboratories:

(1) A *Cabinet Laboratory* where all handling of agents must be performed in a Class III BSC.

(2) A *Suit Laboratory* where personnel must wear a positive pressure 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. All persons leaving the laboratory must be required to take a personal body shower.

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. 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. 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. Plasticware 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.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.

8. Decontaminate all wastes before removal from the laboratory by an effective and validated method.

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 child-bearing 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 in accordance with institutional policies.

   Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes should be 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. Laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves, must be used by all personnel entering the laboratory. 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, 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 service 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 must establish policies and procedures describing the 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 or 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 are interlocked in a manner that prevents opening of the outer door unless the autoclave has been operated through a decontamination cycle. The doors of a fumigation chamber must be secured in a manner that prevents opening of the outer door unless the fumigation chamber has been operated through a fumigation 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 facility.

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 facility 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 leaks periodically 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. Protective laboratory clothing with a solid-front such as tie-back or wrap-around gowns, scrub suits, or coveralls must be worn by workers when in the laboratory. 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 before being laundered.

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. Gloves must be worn to protect against breaks or tears in the cabinet gloves. 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 suit ventilated with a life support system.

   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 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 recommendations.

2. Protective laboratory clothing such as scrub suits must be worn by workers before entering the room used for donning positive pressure suits. All protective clothing must be removed in the dirty side change room before entering the personal shower. Reusable laboratory clothing must be autoclaved before being laundered.

3. Inner 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 personal shower. Dispose of used gloves with other contaminated waste.

4. Decontamination of outer suit gloves is performed during 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 prior to 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, and security systems should be on an uninterrupted power supply (UPS).

   A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom/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 and the inner (dirty) change room 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 liquids and 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, plumbing or otherwise that penetrate the laboratory walls, floors, ceiling, plumbing or otherwise, must 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 of the biological agents in use.

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 should 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 each individual laboratory system is isolated by gas tight dampers and HEPA filters.

   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/directional airflow 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.

   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 or laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must to 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. BSCs 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.

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 primary wall. This bioseal must be durable and airtight. 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 over-pressurization cannot release unfiltered air or steam exposed to infectious material 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 access/egress must be considered.

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 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 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, 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.

2. Sinks inside the suit laboratory should be placed near procedure areas and contain traps 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 liquids and 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 and other service lines must be protected by two HEPA filters in series and have protection against insect and animal intrusion.

Services, plumbing or otherwise that penetrate the laboratory walls, floors, ceiling, plumbing or otherwise, must 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 laboratory must be performed using a validated gaseous or vapor method when there have been significant changes in laboratory usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.

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 should 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 each individual laboratory system is isolated by gas tight dampers and HEPA filters.

   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/directional airflow 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.

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 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 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 primary wall. This bioseal must be durable and airtight. 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 over-pressurization cannot release unfiltered air or steam exposed to infectious material 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 access/egress should be considered.

TABLE 1
SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Open bench and sink required
2	• Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs*: • Laboratory coats; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents PPEs*: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text

* PPE – Personal Protective Equipment

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 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 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 chapter 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 (ABSL-3Ag). 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 chapter. 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 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 Chapter 2).

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 emergency situations.

   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. 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. 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 child-bearing 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 required 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 use should only be done in designated areas and are not permitted in animal or procedure rooms.

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. Needles and syringes or other sharp instruments are limited to use in the animal facility when 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. Plasticware 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. Special containment devices or equipment may not be required as determined by appropriate risk assessment.

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

   Protective laboratory coats, gowns, or uniforms are recommended 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.

3. 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 the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, faceshields, etc.) as needed.

4. Gloves should be 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, integrity has been 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 prohibits 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 or 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.

   It is recommended that penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, 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.

   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 should 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.

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) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should 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. 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 emergency situations.

   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 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.

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

3. 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. 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 child-bearing 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 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 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 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 required 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 use should only be done in designated areas and are not permitted in animal or procedure rooms.

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. Needles and syringes or other sharp instruments are limited to use in the animal facility when 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; it should be removed using a brush and dustpan, tongs, or forceps. Plasticware 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 containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

    Decontaminate of 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.

   Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).

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

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

   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 contain 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 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.

5. 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 or splashes. 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 larger cages placed in inward flow ventilated enclosures 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 anticipated splashes/ 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 the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, faceshields, etc.) as needed. Respiratory protection is worn based upon risk assessment.

4. Gloves should be 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, integrity has been 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 prohibits 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 or 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 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.

   Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, 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 should 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 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 should 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 a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. Correct performance of the BSCs should be recertified at least once a year.

    All BSCs should be used according to manufacturer's recommendation, to protect the worker and avoid creating a hazardous environment from volatile chemical 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 considered 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.

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 emergency situations.

   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 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 and special 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. 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. 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 child-bearing 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 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 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 required to prevent contamination of personal clothing.

   Gloves are worn to prevent skin contact with contaminated, infectious/ and 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 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 use should only be done in designated areas and are not permitted in animal or procedure rooms.

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. Needles and syringes or other sharp instruments are limited to use in the animal facility when 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; it should be removed using a brush and dustpan, tongs, or forceps**.** Plasticware 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 containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

    Decontaminate of 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 infected animals or the generations 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.

   Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).

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 when operational malfunctions occur.

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 should 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) before removal from the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method.

   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. Autoclaving of content prior to incineration is recommended.

6. Equipment, cages, and racks should be handled in 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 through the use of 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.

   Protective clothing such as uniforms or scrub suits is worn by personnel within the animal facility. 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. Appropriate eye, face and respiratory protection are worn by all personnel entering areas where infectious materials and/or animals are housed or are manipulated. 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 should be 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, integrity has been 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 prohibits 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 or 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.

   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, to include openings around ducts, doors and door frames, 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 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, 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 to 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 should 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. 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, or the exhaust must be HEPA-filtered.

   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 and confirms directional inward airflow be provided at the animal room entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the animal spaces. Audible 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 its 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 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 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' recommendations.

    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 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 or 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 life-threatening disease, aerosol transmission, or 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 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. Access to the animal facility within the BSL-4 laboratory is controlled by the animal facility director and/or laboratory supervisor in accordance with institutional policies.

There are two models for ABSL-4 laboratories:

(1) A *Cabinet Laboratory* where all handling of agents, infected animals and housing of infected animals must be performed in Class III BSCs (See Appendix A).

(2) A *Suit Laboratory* where 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; 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 emergency situations 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 staffs are informed of potential occupational hazards within the animal facility including those associated with 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 polices to prevent hazardous exposures to personnel who manipulate and care for animals.

2. A complete clothing change is required In the ABSL-4 operation. Protective clothing such as uniforms or scrub suits are worn by personnel within the animal facility.

   All persons leaving the BSL-4/ABSL-4 laboratory are required to take a personal body shower.

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. 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.

   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. Needles and syringes or other sharp instruments are limited for use in the animal facility when 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 and placed 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**.** Plasticware should be substituted for glassware whenever possible.

   e. Equipment containing sharp edges and corners should be avoided.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

   Procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets, or other physical containment devices. When procedures can not be performed in a BSC, alternate containment equipment should be used.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

   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 animal facility director, laboratory supervisor, institutional management and

appropriate facility safety personnel. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

8. Decontaminate all wastes (including animal tissues, carcasses, and contaminated bedding) and other materials before removal from the-ABSL-4 laboratory by an effective and validated method. Laboratory clothing should be decontaminated before laundering.

   Supplies and materials needed in the facility must be brought in through a double-door autoclave, fumigation chamber, or airlock. Supplies and materials that are not brought into the ABSL-4 laboratory through the change room must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. Containment should be maintained at all times. After securing the outer doors, personnel within the areas where infectious materials and/or animals are housed or are manipulated 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.

   Only necessary equipment and supplies should be taken inside the ABSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal. Consideration should be given to means for decontaminating routine husbandry equipment and sensitive electronic and medical equipment.

   The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave has been operated through a decontamination cycle or the fumigation chamber has been decontaminated.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory and the animal room/s 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 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 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]

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 child-bearing 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.

12. 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.

B. ***Special Practices***

1. All persons entering the ABSL-4 laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

   Only persons whose presence in the laboratory or individual animal rooms is required for scientific or support purposes should be 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 ABSL-4 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. Laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves, must be used by all personnel entering the laboratory.

   All persons leaving the ABSL-4 laboratory are required to 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 or disposal.

   After the laboratory has been completely decontaminated by validated method, necessary staff may enter and exit the laboratory without following the clothing change and shower requirements described above.

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 child-bearing 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.

2. Animal facility personnel and support staff must be provided 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-acquired illnesses. An essential adjunct to an occupational medical system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired illnesses.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

4. The animal facility supervisor is responsible for ensuring that animal personnel:

    a. Receive appropriate training in the practices and operations specific to the animal facility, such as animal husbandry procedures, potential hazards present, manipulations of infectious agents, necessary precautions to prevent potential exposures.

    b. Demonstrate high proficiency in standard and special microbiological practices, and techniques before entering the ABSL-4 facility or working with agents requiring BSL-4 containment.

    c. Receive annual updates and additional training when procedure or policy changes occur. Records are maintained for all hazard evaluations and employee training.

5. Removal of biological materials that are to remain in a viable or intact state from the ABSL-4 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.

6. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination. Equipment, cages, and racks

should be handled in manner that minimizes contamination of other areas. Cages are autoclaved or thoroughly decontaminated before they are cleaned and washed.

a. All equipment and contaminated materials must be decontaminated before removal from the animal facility. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the animal facility.

b. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as gaseous or vapor method in an airlock or chamber designed for this purpose.

c. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory. Spills and accidents of potentially infectious materials must be immediately reported to the animal facility and laboratory supervisors or personnel designated by the institution.

7. The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave/decontamination chamber has been operated through a decontamination cycle or the fumigation chamber has been decontaminated.

8. Daily inspections of essential containment and life support systems must be completed before laboratory work is initiated to ensure that the laboratory and animal facilities are operating according to its established parameters.

9. 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-ABSL-4 laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel according to institutional policies.

10. Based on site-specific risk assessment, personnel assigned to work with infected animals may be required to work in pairs. Procedures to reduce possible worker exposure must be instituted, such as use of squeeze cages, working only with anesthetized animals, or other appropriate practices.

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

Cabinet Laboratory

1. All manipulations of infectious animals and materials within the laboratory must be conducted in the Class III BSC.

   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 leaks periodically 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 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.

   The Class III cabinet must be certified at least annually.

Restraint devices and practices that reduce the risk of exposure during animal manipulations should be used where practicable (e.g., physical restraint devices, chemical restraint medications, mesh or Kevlar gloves, etc.).

2. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls must be worn by workers when in the laboratory. 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 laboratory clothing must be autoclaved before being laundered.

3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eye glasses must be decontaminated before removal thought the personal body shower.

4. Gloves must be worn to protect against breaks or tears in the cabinet gloves. 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 waste.

Suit Laboratory

1. Infected animals should be housed in a primary containment system (such as open cages placed in ventilated enclosures, solid wall and bottom cages covered with filter bonnets and opened in laminar flow hoods, or other equivalent primary containment systems).

   All procedures must be conducted by personnel wearing a one-piece positive pressure suit ventilated with a life support system.

   All manipulations of potentially infectious agents must be performed within a Class II BSC or other primary barrier system. Infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

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

   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.

2. Protective laboratory clothing such as scrub suits must be worn by workers before entering the room used for donning positive pressure suits. All protective clothing must be removed in the dirty side change room before entering the personal shower. Reusable laboratory clothing must be autoclaved before being laundered.

3. Inner 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 personal shower. Dispose of used gloves with other contaminated waste.

4. Decontamination of outer suit gloves is performed during operations to remove gross contamination and minimize further contamination of the laboratory.

D. *Laboratory Facilities (Secondary Barriers)*

Cabinet Laboratory

1. The ABSL-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 ABSL-4 facility must be arranged to ensure sequential passage through an inner (dirty) change area, personal shower and outer (clean) change room prior to 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, and security systems should be on an uninterrupted power supply (UPS).

   A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom/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 doors of the cabinet room(s) and the inner change rooms. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC and the inner (dirty) change room 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 liquids and 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.

All drains in ABSL-4 laboratory area floor must be connected directly to the liquid waste decontamination system.

Services that penetrate the walls, floors or ceiling, plumbing or otherwise, must ensure that no backflow from the laboratory occurs. Services must be sealed and be provided with redundant backflow prevention. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and are 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 of the biological agents in use.

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 should 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 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 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 each individual laboratory system is isolated by gas tight dampers and HEPA filters.

   The supply and exhaust components of the ventilation system must be designed to maintain the ABSL-4 laboratory at negative pressure to surrounding areas and provide differential pressure/directional airflow 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.

   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 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. 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.

    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 though shall be limited to those individuals authorized to be in the ABSL-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 as required by institutional policy.

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

13. A double-door autoclave must be provided for decontaminating waste or other materials passing out of the cabinet room. Autoclaves that open outside of the laboratory must be sealed to the wall. This bioseal must be durable, airtight, and sealed to the wall. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is 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 can only be opened after the autoclave 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 over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The ABSL-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 ABSL-4 laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress must be considered.

Suit Laboratory

1. The ABSL-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 sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

   Entry to this area must be through an airlock fitted with airtight doors. Personnel who enter this area must wear a positive pressure suit ventilated by a life support system with HEPA filtered breathing air. The breathing air system must have redundant compressors, failure alarms and emergency backup system.

   A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the ABSL-4 laboratory. In the event of an emergency exit or failure of chemical shower, 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, 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.

2. Sinks inside the-ABSL-4 laboratory should be placed near procedure areas and contain traps and be connected to the wastewater decontamination system.

3. Walls, floors, and ceilings of the ABSL-4 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 liquids and 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 and other service lines must be protected by two HEPA filters in series and have protection against insect and animal intrusion.

Services, plumbing or otherwise that penetrate the laboratory walls, floors, ceiling, plumbing or otherwise, must 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 laboratory must be performed using a validated gaseous or vapor method when there have been significant changes in laboratory usage, before major renovations or maintenance shut downs, and in other situations, as determined by 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, decontamination and unencumbered movement of personnel. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated. Sharp edges and corners should be avoided.

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 ABSL-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 each individual laboratory system is isolated by gas tight dampers and HEPA filters.

   The supply and exhaust components of the ventilation system must be designed to maintain the BSL-4/ABSL-4 laboratory at negative pressure to surrounding areas and provide differential pressure/directional airflow 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.

Supply air to the ABSL-4 laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the BSL-4/ABSL-4 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 areas where infectious materials and/or animals are housed or are manipulated in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings are 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 ABSL-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 as 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 wall through which the autoclave passes. This bioseal must be durable and airtight. 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.

    The size of the autoclave should be sufficient to accommodate the intended usage, equipment size, and potential future increases in cage size. Autoclaves should facilitate isolation for routine servicing.

    Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material 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. Verification criteria should be modified as necessary by operational experience.

    Consider placing ABSL-4 areas away from exterior walls of buildings to minimize the impact from the outside environmental and temperatures.

15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress should be considered.

REFERENCES

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3. National Research Council; Institute for Laboratory Animal Research. Occupational health and safety in the care of research animals. Washington, DC: National Academy Press; 1996.

4. National Research Council; Institute for Laboratory Animal Research. Occupational health and safety in the care and use of nonhuman primates. Washington, DC: National Academy Press; 2003.

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TABLE 1
SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR ACTIVITIES IN WHICH EXPERIMENTALLY OR NATURALLY INFECTED VERTBRATE ANIMALS ARE USED.

ABSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	Standard animal facility: • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available
2	• Associated with human disease • Hazard: percutaneous exposure, ingestion, mucous membrane exposure.	ABSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual • Decontamination of all infectious wastes and of animal cages prior to washing	ABSL-1 equipment plus primary barriers: • Containment equipment appropriate for animal species PPEs*: • Laboratory coats, gloves, face and respiratory protection as needed	ABSL-1 plus: • Autoclave available • Hand washing sink available • Mechanical cage washer recommended
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious health effects	ABSL-2 practice plus: • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed	ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: • Appropriate respiratory protection	ABSL-2 facility plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility
4	• Dangerous/exotic agents that pose high risk of life threatening disease • Aerosol transmission, or related agents with unknown risk of transmission	ABSL-3 practices plus: • Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting • All wastes are decontaminated before removal from the facility	ABSL-3 equipment plus: • Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities	ABSL-3 facility plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum and decontamination systems • Other requirements outlined in the text

* PPE – Personal Protective Equipment

Section VI

Principles of Laboratory Biosecurity

Since the publication of the 4th edition of BMBL manual in 1999, significant events have brought national and international scrutiny to the area of laboratory security. These events, including the anthrax attacks on U.S. citizens in October 2001 and the subsequent expansion of the United States Select Agent regulations in December 2003, have led scientists, laboratory managers, security specialists, biosafety professionals, and other scientific and institutional leaders to consider the need for developing, implementing and/or improving the security of biological agents and toxins within their facilities. Appendix F of BMBL 4th edition provided a brief outline of issues to consider in developing a security plan for biological agents and toxins capable of serious or fatal illness to humans or animals. In December 2002, Appendix F was updated and re-released as a security and emergency response guidance for laboratories working with Select Agents.[1] A further updated and revised Appendix F is included in the 5th edition of BMBL and is focused exclusively on Select Agent Laboratories and includes current references for the USDA and CDC Select Agent Programs.

This chapter describes laboratory biosecurity planning for microbiological laboratories. As indicated below, laboratories with good biosafety programs already fulfill many of the basic requirements for security of biological materials. For laboratories not handling select agents, the access controls and training requirements specified for BSL-2 and BSL-3 in BMBL may provide sufficient security for the materials being studied. Security assessments and additional security measures should be considered when select agents, other agents of high public health and agriculture concern, or agents of high commercial value such as patented vaccine candidates, are introduced into the laboratory.

The recommendations presented in this chapter are advisory. Excluding the Select Agent Regulations, there is no current federal requirement for the development of a biosecurity program. However, the application of these principles and the assessment process may enhance overall laboratory management. Laboratories that fall under the Select Agent regulations should consult Appendix F (42 CFR part 73; 7 CFR 331 and 9 CFR 121).[4,5,6]

The term “biosecurity” has multiple definitions. In the animal industry, the term biosecurity relates to the protection of an animal colony from microbial contamination. In some countries, the term biosecurity is used in place of the term biosafety. For the purposes of this chapter the term “biosecurity” will refer to the protection of microbial agents from loss, theft, diversion or intentional misuse. This is consistent with current WHO and American Biological Safety Association (ABSA) usage of this term.[1,2,3]

Security is not a new concept in biological research and medical laboratories. Several of the security measures discussed in this chapter are embedded in the biosafety levels that serve as the foundation for good laboratory practices throughout the biological

laboratory community. Most biomedical and microbiological laboratories do not have select agents or toxins, yet maintain control over and account for research materials, protect relevant sensitive information, and work in facilities with access controls commensurate with the potential public health and economic impact of the biological agents in their collections. These measures are in place in most laboratories that apply good laboratory management practices and have appropriate biosafety programs.

BIOSAFETY AND BIOSECURITY

Biosafety and biosecurity are related, but not identical, concepts. Biosafety programs reduce or eliminate exposure of individuals and the environment to potentially hazardous biological agents. Biosafety is achieved by implementing various degrees of laboratory control and containment, through laboratory design and access restrictions, personnel expertise and training, use of containment equipment, and safe methods of managing infectious materials in a laboratory setting.

The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information. This is accomplished by limiting access to facilities, research materials and information. While the objectives are different, biosafety and biosecurity measures are usually complementary.

Biosafety and biosecurity programs share common components. Both are based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements, material transfer documentation, training, emergency planning, and program management.

Biosafety and biosecurity program risk assessments are performed to determine the appropriate levels of controls within each program. Biosafety looks at appropriate laboratory procedures and practices necessary to prevent exposures and occupationally-acquired infections, while biosecurity addresses procedures and practices to ensure that biological materials and relevant sensitive information remain secure.

Both programs assess personnel qualifications. The biosafety program ensures that staff are qualified to perform their jobs safely through training and documentation of technical expertise. Staff must exhibit the appropriate level of professional responsibility for management of research materials by adherence to appropriate materials management procedures. Biosafety practices require laboratory access to be limited when work is in progress. Biosecurity practices ensure that access to the laboratory facility and biological materials are limited and controlled as necessary. An inventory or material management process for control and tracking of biological stocks or other sensitive materials is also a component of both programs. For biosafety, the shipment of infectious biological materials must adhere to safe packaging, containment and appropriate transport procedures, while biosecurity ensures that transfers are controlled, tracked and documented commensurate with the potential risks. Both programs must engage laboratory personnel in the development of practices and procedures that fulfill the

biosafety and biosecurity program objectives but that do not hinder research or clinical/diagnostic activities. The success of both of these programs hinges on a laboratory culture that understands and accepts the rationale for biosafety and biosecurity programs and the corresponding management oversight.

In some cases, biosecurity practices may conflict with biosafety practices, requiring personnel and management to devise policies that accommodate both sets of objectives. For example, signage may present a conflict between the two programs. Standard biosafety practice requires that signage be posted on laboratory doors to alert people to the hazards that may be present within the laboratory. The biohazard sign normally includes the name of the agent, specific hazards associated with the use or handling of the agent and contact information for the investigator. These practices may conflict with security objectives. Therefore, biosafety and biosecurity considerations must be balanced and proportional to the identified risks when developing institutional policies.

Designing a biosecurity program that does not jeopardize laboratory operations or interfere with the conduct of research requires a familiarity with microbiology and the materials that require protection. Protecting pathogens and other sensitive biological materials while preserving the free exchange of research materials and information may present significant institutional challenges. Therefore, a combination or tiered approach to protecting biological materials, commensurate with the identified risks, often provides the best resolution to conflicts that may arise. However, in the absence of legal requirements for a biosecurity program, the health and safety of laboratory personnel and the surrounding environment should take precedence over biosecurity concerns.

RISK MANAGEMENT METHODOLOGY

A risk management methodology can be used to identify the need for a biosecurity program. A risk management approach to laboratory biosecurity 1) establishes which, if any, agents require biosecurity measures to prevent loss, theft, diversion, or intentional misuse, and 2) ensures that the protective measures provided, and the costs associated with that protection, are proportional to the risk. The need for a biosecurity program should be based on the possible impact of the theft, loss, diversion, or intentional misuse of the materials, recognizing that different agents and toxins will pose different levels of risk. Resources are not infinite. Biosecurity policies and procedures should not seek to protect against every conceivable risk. The risks need to be identified, prioritized and resources allocated based on that prioritization. Not all institutions will rank the same agent at the same risk level. Risk management methodology takes into consideration available institutional resources and the risk tolerance of the institution.

Developing a Biosecurity Program

Management, researchers and laboratory supervisors must be committed to being responsible stewards of infectious agents and toxins. Development of a biosecurity

program should be a collaborative process involving all stakeholders. The stakeholders include but are not be limited to: senior management, scientific staff, human resource officials, information technology staff, and safety, security and engineering officials. The involvement of organizations and/or personnel responsible for a facility's overall security is critical because many potential biosecurity measures may already be in place as part of an existing safety or security program. This coordinated approach is critical in ensuring that the biosecurity program provides reasonable, timely and cost effective solutions addressing the identified security risks without unduly affecting the scientific or business enterprise or provision of clinical and/or diagnostic services.

The need for a biosecurity program should reflect sound risk management practices based on a site-specific risk assessment. A biosecurity risk assessment should analyze the probability and consequences of loss, theft and potential misuse of pathogens and toxins.[7] Most importantly, the biosecurity risk assessment should be used as the basis for making risk management decisions.

Example Guidance: A Biosecurity Risk Assessment and Management Process

Different models exist regarding biosecurity risk assessment. Most models share common components such as asset identification, threat, vulnerability and mitigation. What follows is one example of how a biosecurity risk assessment may be conducted. In this example, the entire risk assessment and risk management process may be divided into five main steps, each of which can be further subdivided: 1) identify and prioritize biologicals and/or toxins; 2) identify and prioritize the adversary/threat to biologicals and/or toxins; 3) analyze the risk of specific security scenarios; 4) design and develop an overall risk management program; 5) regularly evaluate the institution's risk posture and protection objectives. Example guidance for these five steps is provided below.

Step 1: Identify and Prioritize Biological Materials

- Identify the biological materials that exist at the institution, form of the material, location and quantities, including non-replicating materials (i.e., toxins).
- Evaluate the potential for misuse of these biologic materials.
- Evaluate the consequences of misuse of these biologic materials.
- Prioritize the biologic materials based on the consequences of misuse (i.e., risk of malicious use).

At this point, an institution may find that none of its biologic materials merit the development and implementation of a separate biosecurity program or the existing security at the facility is adequate. In this event, no additional steps would need to be completed.

Step 2: Identify and Prioritize the Threat to Biological Materials

- Identify the types of "Insiders" who may pose a threat to the biologic materials at the institution.
- Identify the types of "Outsiders" (if any) who may pose a threat to the biologic materials at the institution.
- Evaluate the motive, means, and opportunity of these various potential adversaries.

Step 3: Analyze the Risk of Specific Security Scenarios

- Develop a list of possible biosecurity scenarios, or undesired events that could occur at the institution (each scenario is a combination of an agent, an adversary, and an action). Consider:
    - access to the agent within your laboratory;
    - how the undesired event could occur;
    - protective measures in place to prevent occurrence;
    - how the existing protection measures could be breached (i.e., vulnerabilities).
- Evaluate the probability of each scenario materializing (i.e., the likelihood) and its associated consequences. Assumptions include:
    - although a wide range of threats are possible, certain threats are more probable than others;
    - all agents/assets are not equally attractive to an adversary;
    - valid and credible threats, existing precautions, and the potential need for select enhanced precautions are considered.
- Prioritize or rank the scenarios by risk for review by management.

Step 4: Develop an Overall Risk Management Program

- Management commits to oversight, implementation, training and maintenance of the biosecurity program.

- Management develops a biosecurity risk statement, documenting which biosecurity scenarios represent an unacceptable risk and must be mitigated versus those risks appropriately handled through existing protection controls.

- Management develops a biosecurity plan to describe how the institution will mitigate those unacceptable risks including:

    - a written security plan, standard operating procedures, and incident response plans;

    - written protocols for employee training on potential hazards, the biosecurity program and incident response plans.

- Management ensures necessary resources to achieve the protection measures documented in the biosecurity plan.

Step 5: Reevaluate the Institution's Risk Posture and Protection Objectives

- Management regularly reevaluates and makes necessary modifications to the:

    - biosecurity risk statement;

    - biosecurity risk assessment process;

    - the institution's biosecurity program/plan;

    - the institution's biosecurity systems.

- Management assures the daily implementation, training and annual re-evaluation of the security program.

ELEMENTS OF A BIOSECURITY PROGRAM

Many facilities may determine that existing safety and security programs provide adequate mitigation for the security concerns identified through biosecurity risk assessment. This section offers examples and suggestions for components of a biosecurity program should the risk assessment reveal that further protections may be warranted. Program components should be site-specific and based upon organizational threat/vulnerability assessment and as determined appropriate by facility management. Elements discussed below should be implemented, as needed, based upon the risk assessment process. They should not be construed as "minimum requirements" or "minimum standards" for a biosecurity program.

Program Management

If a biosecurity plan is implemented, institutional management must support the biosecurity program. Appropriate authority must be delegated for implementation and the necessary resources provided to assure program goals are being met. An organizational structure for the biosecurity program that clearly defines the chain of command, roles, and responsibilities should be distributed to the staff. Program management should ensure that biosecurity plans are created, exercised, and revised as needed. The biosecurity program should be integrated into relevant institutional policies and plans.

Physical Security – Access Control and Monitoring

The physical security elements of a laboratory biosecurity program are intended to prevent the removal of assets for non-official purposes. An evaluation of the physical security measures should include a thorough review of the building and premises, the laboratories and biological material storage areas. Many requirements for a biosecurity plan may already exist in a facility's overall security plan.

Access should be limited to authorized and designated employees based on the need to enter sensitive areas. Methods for limiting access could be as simple as locking doors or having a card key system in place. Evaluations on the levels of access should consider all facets of the laboratory's operations and programs (e.g., laboratory entrance requirements, freezer access, etc.). The need for entry by visitors, laboratory workers, management officials, students, cleaning/maintenance staff and emergency response personnel should be considered.

Personnel Management

Personnel management includes identifying the roles and responsibilities for employees who handle, use, store and transport dangerous pathogens and/or other important assets. The effectiveness of a biosecurity program against identified threats depends, first and foremost, on the integrity of those individuals who have access to pathogens, toxins, sensitive information and/or other assets. Employee screening policies and procedures are used to help evaluate these individuals. Policies should be developed for personnel and visitor identification, visitor management, access procedures, and reporting of security incidents.

Inventory and Accountability

Material accountability procedures should be established to track the inventory, storage, use, transfer and destruction of dangerous biological materials and assets when no longer needed. The objective is to know what agents exist at a facility, where they are located, and who is responsible for them. To achieve this, management should define: 1) the materials (or forms of materials) subject to accountability measures; 2) records to be maintained, update intervals and timelines for record maintenance; 3) operating procedures associated with inventory maintenance (e.g., how material is identified, where it can be stored, used, etc.); and 4) documentation and reporting requirements.

It is important to emphasize that microbiological agents are capable of replication and are often expanded to accommodate the nature of the work involving their use. Therefore, knowing the exact “working” quantity of organisms at any given time may be impractical. Depending on the risks associated with a pathogen or toxin, management can designate an accountable individual who is knowledgeable about the materials in use and responsible for security of the materials under his or her control.

Information Security

Policies should be established for handling sensitive information associated with the biosecurity program. For the purpose of these policies "sensitive information" is that which is related to the security of pathogens and toxins, or other critical infrastructure information. Examples of sensitive information may include facility security plans, access control codes, agent inventories and storage locations. Discussion of information security in this section does not pertain to information which has been designated “classified” by the United States pursuant to Executive Order 12958, as amended, and is governed by United States law or to research-related information which is typically unregulated or unrestricted through the peer review and approval processes.

The objective of an information security program is to protect information from unauthorized release and ensure that the appropriate level of confidentiality is preserved. Facilities should develop policies that govern the identification, marking and handling of sensitive information. The information security program should be tailored to meet the needs of the business environment, support the mission of the organization, and mitigate the identified threats. It is critical that access to sensitive information be controlled. Policies for properly identifying and securing sensitive information including electronic files and removable electronic media (e.g., CDs, computer drives, etc.) should be developed.

Transport of Biological Agents

Material transport policies should include accountability measures for the movement of materials within an institution (e.g., between laboratories, during shipping and receiving activities, etc.) and outside of the facility (e.g., between institutions or locations). Transport policies should address the need for appropriate documentation and material accountability and control procedures for pathogens in transit between locations. Transport security measures should be instituted to ensure that appropriate authorizations have been received and that adequate communication between facilities has occurred before, during, and after transport of pathogens or other potentially hazardous biological materials. Personnel should be adequately trained and familiar with regulatory and institutional procedures for proper containment, packaging, labeling, documentation and transport of biological materials.

Accident, Injury and Incident Response Plans

Laboratory security policies should consider situations that may require emergency responders or public safety personnel to enter the facility in response to an accident, injury or other safety issue or security threat. The preservation of human life, the safety and health of laboratory employees and the surrounding community must take precedence in an emergency over biosecurity concerns. Facilities are encouraged to coordinate with medical, fire, police and other emergency officials when preparing emergency and security breach response plans. Standard Operation Procedures (SOPs) should be developed that minimize the potential exposure of responding personnel to potentially hazardous biological materials. Laboratory emergency response plans should be integrated with relevant facility-wide or site specific security plans. These plans should also consider such adverse events as bomb threats, natural disasters and severe weather, power outages, and other facility emergencies that may introduce security threats.

Reporting and Communication

Communication is an important aspect of a biosecurity program. A "chain-of-notification" should be established in advance of an actual event. This communication chain should include laboratory and program officials, institution management, and any relevant regulatory or public authorities. The roles and responsibilities of all involved officials and programs should be clearly defined. Policies should address the reporting and investigation of potential security breaches (e.g., missing biological agents, unusual or threatening phone calls, unauthorized personnel in restricted areas, etc.).

Training and Practice Drills

Biosecurity training is essential for the successful implementation of a biosecurity program. Program management should establish training programs that inform and educate individuals regarding their responsibilities within the laboratory and the institution. Practice drills should address a variety of scenarios such as loss or theft of materials, emergency response to accidents and injuries, incident reporting and identification of and response to security breaches. These scenarios may be incorporated into existing emergency response drills such as fire drills or building evacuation drills associated with bomb threats. Incorporating biosecurity measures into existing procedures and response plans often provides efficient use of resources, saves time and can minimize confusion in an emergency situation.

Security Updates and Reevaluations

The biosecurity risk assessment and program should be reviewed and updated routinely and following any biosecurity-related incident. Reevaluation is a necessary and on-going process in the dynamic environments of today's biomedical and research laboratories. Biosecurity program managers should develop and conduct biosecurity program audits and implement corrective actions as needed. Audit results and corrective actions should be documented. Records should be maintained by the appropriate program officials.

Select Agents

If an entity possesses, uses or transfer select agents, it must comply with all requirements of the National Select Agent Program. See Appendix F for additional guidance on the CDC and USDA Select Agent Programs (42 CFR part 73; 7 CFR 331 and 9 CFR 121).

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Section VII

Occupational Health and Immunoprophylaxis

The goal of medical support services in a biomedical research setting is to promote a safe and healthy workplace. This is accomplished by limiting opportunities for exposure, promptly detecting and treating exposures, and using information gained from work injuries to further enhance safety precautions. Occupational health and safety in biomedical research settings is a responsibility shared by healthcare providers, safety specialists, principal investigators, employers, and workplace personnel. Optimal worker protection depends on effective, ongoing collaboration among these groups. Supervisors, working with personnel representatives, should describe workers' proposed tasks and responsibilities. First line supervisors and safety professionals should identify the potential worksite health hazards. Principal investigators may serve as subject matter experts. The health provider should design medical support services in consultation with representatives from the institutional environmental health and safety program and the principal investigators. Workers should be fully informed of the available medical support services and encouraged to utilize them. Requisite occupational medical services are described below and expanded discussions of the principles of effective medical support services are available in authoritative texts.[1,2]

Services offered by the medical support team should be designed to be in compliance with United States Department of Labor (DOL), OSHA regulations, patient confidentiality laws, and the Americans with Disabilities Act of 1990.[3-8] Medical support services should be based upon detailed risk assessments and tailored to meet the organization's needs. Risk assessments should define potential hazards and exposures by job responsibility. They should be provided for all personnel regardless of employment status. Contracted workers, students, and visitors should be provided occupational medical care by their employer or sponsor equivalent to that provided by the host institution for exposures, injuries, or other emergencies experienced at the worksite.

Occupational medical services may be provided through a variety of arrangements (e.g., in-house or community based) as long as the service is readily available and allows timely, appropriate evaluation and treatment. The interaction between worker, healthcare provider and employer may be complex, such as a contract worker who uses his own medical provider or uses contract medical services. Thus, plans for providing medical support for workers should be completed before work actually begins. The medical provider must be knowledgeable about the nature of potential health risks in the work environment and have access to expert consultation.

Prevention is the most effective approach to managing biohazards. Prospective workers should be educated about the biohazards to which they may be occupationally exposed, the types of exposures that place their health at risk, the nature and significance of such risks, as well as the appropriate first aid and follow up for potential exposures.

That information should be reinforced annually, at the time of any significant change in job responsibility, and following recognized and suspected exposures.[9-11]

Medical support services for biomedical research facilities should be evaluated annually. Joint annual review of occupational injury and illness reports by healthcare providers and environmental health and safety representatives can assist revision of exposure prevention strategies to minimize occupational health hazards that cannot be eliminated.

OCCUPATIONAL HEALTH SUPPORT SERVICE ELEMENTS

Preplacement Medical Evaluations

Workers who may be exposed to human pathogens should receive a preplacement medical evaluation. Healthcare providers should be cognizant of potential hazards encountered by the worker. A description of the requirements for the position and an understanding of the potential health hazards present in the work environment, provided by the worker's supervisor, should guide the evaluation. The healthcare provider should review the worker's previous and ongoing medical problems, current medications, allergies to medicines, animals, and other environmental proteins, and prior immunizations. With that information, the healthcare provider determines what medical services are indicated to permit the individual to safely assume the duties of the position. Occasionally, it may be useful to review pre-existing medical records to address specific concerns regarding an individual's medical fitness to perform the duties of a specific position. If pre-existing medical records are unavailable or are inadequate, the healthcare provider may need to perform a targeted medical exam. Comprehensive physical examinations are rarely indicated. During the visit, the healthcare provider should inform the worker of potential health hazards in the work area and review steps that should be taken in the event of an accidental exposure. This visit also establishes a link with the medical support services provider.

When occupational exposure to human pathogens is a risk, employers should consider collecting and storing a serum specimen prior to the initiation of work with the agent. It can be used to establish baseline sero-reactivity, should additional blood samples be collected for serological testing subsequent to a recognized or suspected exposure.

Occasionally, it is desirable to determine an individual's vulnerability to infection with specific agents prior to assigning work responsibilities. Some occupational exposures present substantially more hazard to identifiable sub-populations of workers. Immunodeficient workers or non-immune pregnant female workers may experience devastating consequences from exposures that pose a chance of risk to pregnant women with prior immunity and other immunocompetent workers (e.g., cytomegalovirus or toxoplasmosis). Serologic testing should be used to document baseline vulnerability to specific infections to which the worker might be exposed, and non-immune workers should be adequately informed about risks. In specific settings, serologic documentation

that individual workers have pre-existing immunity to specific infections also may be required for the protection of research animals.[10]

Vaccines

Commercial vaccines should be made available to workers to provide protection against infectious agents to which they may be occupationally exposed.[12-16] The Advisory Committee on Immunization Practices (ACIP) provides expert advice to the Secretary of the DHHS, the Assistant Secretary for Health, and the CDC on the most effective means to prevent vaccine-preventable diseases and to increase the safe usage of vaccines and related biological products. The ACIP develops recommendations for the routine administration of vaccines to pediatric and adult populations, and schedules regarding the appropriate periodicity, dosage, and contraindications. The ACIP is the only entity in the federal government that makes such recommendations. They are available on the CDC website (www.cdc.gov).

If the potential consequences of infection are substantial and the protective benefit from immunization is proven, acceptance of such immunization may be a condition for employment. Current, applicable vaccine information statements must be provided whenever a vaccine is administered. Each worker's immunization history should be evaluated for completeness and currency at the time of employment and re-evaluated when the individual is assigned job responsibilities with a new biohazard.

When occupational exposure to highly pathogenic agents is possible and no commercial vaccine is available, it may be appropriate to immunize workers using vaccines or immune serum preparations that are investigational, or for which the specific indication constitutes an off-label use. Use of investigational products, or of licensed products for off-label indications must be accompanied by adequate informed consent outlining the limited availability of information on safety and efficacy. Use of investigational products should occur through Investigational New Drug (IND) protocols providing safety oversight by both the Food and Drug Administration (FDA) and appropriate Institutional Human Subjects Research Protection Committees.[17,18] Recommendation of investigational products as well as of commercial vaccines that are less efficacious, associated with high rates of local or systemic reactions, or that produce increasingly severe reactions with repeated use should be considered carefully. Receipt of such vaccines is rarely justified as a job requirement.

Investigational vaccines for eastern equine encephalomyelitis (EEE) virus, Venezuelan equine encephalitis (VEE) virus, western equine encephalomyelitis (WEE) virus, and Rift Valley fever viruses (RVFV), may be available in limited quantities and administered on-site at the Special Immunization Program, United States Army Medical Research Institute of Infectious Diseases (USAMRIID).

Periodic Medical Evaluations

Routine, periodic medical evaluations generally are not recommended; however, limited periodic medical evaluations or medical clearances targeted to job requirements may occasionally be warranted (e.g., respirator usage).[3] In special circumstances, it may be appropriate to offer periodic laboratory testing to workers with substantial risk of exposure to infectious agents to detect pre-clinical or sub-clinical evidence for an occupationally acquired infection. Before asymptomatic workers without specific exposures are tested for seroreactivity, the benefit of such testing should be justified, plans for further investigation of indeterminate test results should be delineated, and clearly defined criteria for interpretation of results should be developed.

Medical Support For Occupational Illnesses And Injuries

Workers should be encouraged to seek medical evaluation for symptoms that they suspect may be related to infectious agents in their work area, without fear of reprisal. A high index of suspicion for potential occupational exposures should be maintained during any unexplained illness among workers or visitors to worksites containing biohazards. Modes of transmission, as well as the clinical presentation of infections acquired through occupational exposures, may differ markedly from naturally acquired infections. Fatal occupational infections have resulted from apparently trivial exposures. The healthcare provider should have a working understanding of the biohazards present in the workplace and remain alert for subtle evidence of infection and atypical presentations. A close working relationship with the research or clinical program in which the affected employee works is absolutely essential. In the event of injury, consultation between healthcare provider, employee, and the employee's supervisor is required for proper medical management and recordkeeping.

All occupational injuries, including exposures to human pathogens, should be reported to the medical support services provider. Strategies for responding to biohazard exposures should be formulated in advance. Proper post-exposure response is facilitated by exposure-specific protocols that define appropriate first aid, potential post-exposure prophylaxis options, recommended diagnostic tests, and sources of expert medical evaluation. These protocols should address how exposures that occur outside of regular work hours are handled and these protocols should be distributed to potential healthcare providers (e.g., local hospital emergency departments). In exceptional cases, the protocols should be reviewed with state and community public health departments. Emergency medical support training should be provided on a regular basis for both employees and healthcare providers.

The adequacy and timeliness of wound cleansing or other response after an exposure occurs may be the most critical determinant in preventing infection. First aid should be defined, widely promulgated, and immediately available to an injured worker. Barriers to subsequent medical evaluation and treatment should be identified and minimized to facilitate prompt, appropriate care. Laboratory SOPs should include a printed summary of the recommended medical response to specific exposures that can guide immediate response in the work place and that the injured worker can provide to the treating facility. The medical provider's description of the injury should include:

- The potential infectious agent.
- The mechanism and route of exposure (percutaneous, splash to mucous membranes or skin, aerosol, etc.).
- Time and place of the incident.
- Personal protective equipment used at the time of the injury.
- Prior first aid provided (e.g., nature and duration of cleaning and other aid, time that lapsed from exposure to treatment).
- Aspects of the worker's personal medical history relevant to risk of infection or complications of treatment.

First aid should be repeated if the initial adequacy is in question. Healthcare providers must use appropriate barrier precautions to avoid exposure to infectious agents and toxins.

In some instances, it may be possible to prevent or ameliorate illness through post-exposure prophylaxis. Protocols should be developed in advance that clearly identify the situations in which post-exposure prophylaxis are to be considered, the appropriate treatment, and the source of products and expert consultation. Accurate quantification of risk associated with all exposures is not possible, and the decision to administer post-exposure prophylaxis may have to be made quickly and in the absence of confirmatory laboratory testing. Post-exposure regimens may involve off-label use of licensed products (e.g., use of smallpox vaccine for workers exposed to monkeypox) in settings where there is insufficient experience to provide exact guidance on the safety or likely protective efficacy of the prophylactic regimen. Thus, protocols should exist that delineate the circumstances under which it would be appropriate to consider use of each product following exposure, as well as the limits of our understanding of the value of some post-exposure interventions. In these cases, consultations with subject matter experts are especially useful.

Estimating the significance of an exposure may be difficult, despite having established protocols. The clinician may need to make a "best-estimate" based upon knowledge of similar agents, exposure circumstances, and advice received from knowledgeable experts. Appropriate post-exposure prophylactic response is always pathogen- and exposure-dependent, may be host-factor dependent, and may also be influenced by immediate post-exposure management. Before prophylactic treatment is undertaken, confirm the likelihood that an exposure occurred, that prophylaxis is indicated and is not contraindicated by past medical history. Conveying this information to the injured worker requires clear, honest communication. The clinical risk assessment and treatment decision process should be carefully explained, the worker's questions addressed with relevant, preprinted educational materials provided. Prompt treatment

should be provided, with a mutually agreed plan to follow the individual's clinical course.

The applicable workers' compensation claim form should be provided with appropriate explanations for its completion.[19] The supervisor must receive a description of the accident or incident, confirm the circumstances of the injury or exposure and provide relevant advice. The report also should be distributed to all other relevant parties, such as the safety professional. Each incident should receive prompt reconsideration of the initial risk assessment and reevaluation of current strategies to reduce the possibility of future exposures.

Post-exposure serologic testing may be useful, but it is important to determine how information obtained from serologic testing will be interpreted. It is also essential to collect serum specimens at the appropriate interval for a given situation. Assessment of sero-reactivity in exposed workers is most helpful when the results of specimens collected over time can be compared. Ideally specimens collected prior to, at the time of and several weeks following exposure should be tested simultaneously and results compared to assess changes in the pattern of sero-reactivity. Serum collected too early after exposure may fail to react even when infection has occurred, because antibodies have not yet been produced in detectable quantities. When immediate institution of post-exposure prophylaxis may delay seroconversion, or when the agent to which the worker was exposed results in seroconversion completed over months (e.g., retroviruses), testing of specimens collected late after exposure is particularly important.

Testing of a single serum specimen is generally discouraged and can result in misinterpretation of nonspecific sero-reactivity. Evidence of sero-conversion or a significant ($\geq$ 4 fold) increase in titer associated with a compatible clinical syndrome is highly suggestive of acute infection. However, the significance of and appropriate response to sero-conversion in the absence of illness is not always clear. If sero-reactivity is evident in the earliest specimen, it is important to re-test that specimen in tandem with serum specimens archived prior to occupational exposure and/or collected serially over time to investigate whether a change in titer suggestive of new infection can be identified.

In some exposure situations, it may be appropriate to store serially collected serum samples, and to send them for testing as evidence of seroconversion only if symptoms develop that suggest an infection may have occurred (e.g., monkey B virus exposures). Serum collected at the time of employment, and any other specimens not immediately tested should be stored frozen at a temperature of -20^{o} C or lower in a freezer that does not experience freeze-thaw cycles. An inventory system should be established to ensure the accurate and timely retrieval of samples, while protecting patient privacy.

When investigational or other non-commercial assays are utilized, the importance of appropriate controls and the ability to compare serially collected specimens for quantification/characterization of reactivity is increased. The availability of aliquoted samples that allow additional testing may be essential to assist interpretation of

ambiguous results. Caution should be taken to avoid placing more confidence in testing outcomes than can be justified by the nature of the assays.

Occupational Health in the BSL-4 Setting

Work with BSL-4 agents involves special challenges for occupational health. Infections of laboratory staff by such agents may be expected to result in serious or lethal disease for which limited treatment options exist. In addition, BSL-4 agents are frequently geographically exotic to the areas in which high containment labs are located but produce immediate public health concern if infections occur in laboratory staff. Potential (if unlikely) transmission from infected staff into the human or animal populations in the areas surrounding the laboratories may raise such concerns to higher levels. Thus, SOPs for BSL-4 settings require special attention to management of unexplained worker absence, including protocols for monitoring, medical evaluation, work-up, and follow-up of workers with unexplained nonspecific illness. Advance planning for the provision of medical care to workers potentially infected with BSL-4 agents is a fundamental component of an occupational health program for a BSL-4 facility.

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SECTION VIII

Agent Summary Statements

- **Section VIII-A: Bacterial Agents**

Agent: Bacillus anthracis

Bacillus anthracis, a gram-positive, non-hemolytic, and non-motile bacillus, is the etiologic agent of anthrax, an acute bacterial disease of mammals, including humans. Like all members of the genus *Bacillus*, under adverse conditions *B. anthracis* has the ability to produce spores that allow the organism to persist for long periods of time until the return of more favorable conditions. Reports of suspected anthrax outbreaks date back to as early as 1250 BC. The study of anthrax and *B. anthracis* in the 1800s contributed greatly to our general understanding of infectious diseases. Much of Koch's postulates were derived from work on identifying the etiologic agent of anthrax. Louis Pasteur developed the first attenuated live vaccine for anthrax.

Most mammals are susceptible to anthrax; it mostly affects herbivores that ingest spores from contaminated soil and, to a lesser extent, carnivores that scavenge on the carcasses of diseased animals. Anthrax still occurs frequently in parts of central Asia and Africa. In the United States, it occurs sporadically in animals in parts of the West, Midwest and Southwest.

The infectious dose varies greatly from species to species and also is route-dependent. The inhalation anthrax infectious dose (ID) for humans primarily has been extrapolated from inhalation challenges of nonhuman primates (NHP) or studies done in contaminated mills. Estimates vary greatly but the lethal dose$_{50}$ (LD) is likely within the range of 2,500-55,000 spores.[1] It is believed that very few spores (10 or less) are required for cutaneous anthrax.[2]

Occupational Infections

Occupational infections are possible when in contact with contaminated animals, animal products or pure cultures of *B. anthracis*, and may include ranchers, veterinarians and laboratory workers. Numerous cases of laboratory-associated anthrax (primarily cutaneous) have been reported.[3,4] Recent cases include suspected cutaneous anthrax in a laboratory worker in Texas and a cutaneous case in a North Dakota male who disposed of five cows that died of anthrax.[5,6]

Natural Modes of Infection

The clinical forms of anthrax in humans that result from different routes of infection are 1) cutaneous (via broken skin), 2) gastrointestinal (via ingestion), and 3)

inhalation anthrax. Cutaneous anthrax is the most common and readily treatable form of the disease. Inhalation anthrax used to be known as "Woolsorter disease" due to its prevalence in textile mill workers handling wool and other contaminated animal products. While naturally occurring disease is no longer a significant public health problem in the United States, anthrax has become a bioterrorism concern. In 2001, 22 people were diagnosed with anthrax acquired from spores sent through the mail, including 11 cases of inhalation anthrax with five deaths and 11 cutaneous cases.[7]

LABORATORY SAFETY

B. anthracis may be present in blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and rarely, in urine and feces. The primary hazards to laboratory personnel are; direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation and rarely, exposure to infectious aerosols. Efforts should be made to avoid production of aerosols by working with infectious organisms in a BSC. In addition, all centrifugation should be done using aerosol-tight rotors that are opened within the BSC after each run.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. ABSL-2 practices, containment equipment and facilities are recommended for studies utilizing experimentally infected laboratory rodents. BSL-3 practices, containment equipment, and facilities are recommended for work involving production quantities or high concentrations of cultures, screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. Workers who frequently centrifuge *B. anthracis* suspensions should use autoclavable aerosol-tight rotors. In addition, regular routine swabbing specimens for culture should be routinely obtained inside the rotor and rotor lid and, if contaminated, rotors should be autoclaved before re-use.

SPECIAL ISSUES

Vaccines A licensed vaccine for anthrax is available. Guidelines for its use in occupational settings are available from the ACIP.[8,9] Worker vaccination is recommended for activities that present an increased risk for repeated exposures to *B. anthracis* spores including: 1) work involving production quantities with a high potential for aerosol production; 2) handling environmental specimens, especially powders associated with anthrax investigations; 3) performing confirmatory testing for *B. anthracis*, with purified cultures; 4) making repeated entries into known *B. anthracis*-spore-contaminated areas after a terrorist attack; 5) work in other settings in which repeated exposure to aerosolized *B. anthracis* spores might occur. Vaccination is not recommended for workers involved in routine processing of clinical specimens or environmental swabs in general diagnostic laboratories.

Select Agent *B. anthracis* is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A Department of Commerce (DoC) permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Bordetella pertussis

Bordetella pertussis, an exclusively human respiratory pathogen of worldwide distribution, is the etiologic agent of whooping cough or pertussis. The organism is a fastidious, small gram-negative coccobacillus that requires highly specialized culture and transport media for cultivation in the laboratory. Its natural habitat is the human respiratory tract.

Occupational Infections

Occupational transmission of pertussis has been reported, primarily among healthcare workers.[10-16] Outbreaks, including secondary transmission, among workers have been documented in hospitals, long-term care institutions, and laboratories. Nosocomial transmissions have been reported in healthcare settings. Laboratory-acquired pertussis has been documented.[17,18]

Natural Modes of Infection

Pertussis is highly communicable, with person-to-person transmission occurring via aerosolized respiratory secretions containing the organism. The attack rate among susceptible hosts is affected by the frequency, proximity, and time of exposure to infected individuals. Although the number of reported pertussis cases declined by over 99% following the introduction of vaccination programs in the 1940s, the 3- to 4-year cycles of cases have continued into the post-vaccination era.[19-21]

LABORATORY SAFETY

The agent may be present in high levels in respiratory secretions, and may be found in other clinical material, such as blood and lung tissue in its infrequent manifestation of septicemia and pneumonia, respectively.[22,23] Because the natural mode of transmission is via the respiratory route, aerosol generation during the manipulation of cultures and contaminated clinical specimens generates the greatest potential hazard.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical

material and cultures. ABSL-2 practices and containment equipment should be employed for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups or safety centrifuges should be used for activities likely to generate potentially infectious aerosols. BSL-3 practices, containment equipment, and facilities are appropriate for production operations.

SPECIAL ISSUES

Vaccines Pertussis vaccines are available but are not currently approved or recommended for use in persons over six years of age. Because this recommendation may change in the near future, the reader is advised to review the current recommendations of the ACIP published in the Morbidity and Mortality Weekly Report (MMWR) and at the CDC Vaccines and Immunizations website for the latest recommendations for adolescents and adults.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Brucella species

The genus *Brucella* consists of slow-growing, very small gram-negative coccobacilli whose natural hosts are mammals. Seven *Brucella* species have been described using epidemiologic and biological characteristics, although at the genetic level all brucellae are closely related. *B. melitensis* (natural host: sheep/goats), *B suis* (natural host: swine), *B. abortus* (natural host: cattle), *B. canis* (natural host: dogs), and *B.* "*maris*" (natural host: marine mammals) have caused illness in humans exposed to the organism including laboratory personnel.[24,25] Hypersensitivity to *Brucella* antigens is a potential but rare hazard to laboratory personnel. Occasional hypersensitivity reactions to *Brucella* antigens occur in workers exposed to experimentally and naturally infected animals or their tissues.

Occupational Infections

Brucellosis has been one of the most frequently reported laboratory infections in the past and cases continue to occur.[4,26-28] Airborne and mucocutaneous exposures can produce LAI. Accidental self-inoculation with vaccine strains is an occupational hazard for veterinarians.

Natural Modes of Infection

Brucellosis (undulant fever, Malta fever, Mediterranean fever) is a zoonotic disease of worldwide occurrence. Mammals, particularly cattle, goats, swine, and sheep act as reservoirs for brucellae. Multiple routes of transmission have been identified,

including direct contact with infected animal tissues or products, ingestion of contaminated milk, and airborne exposure in pens and stables.

LABORATORY SAFETY

Brucella infects the blood and a wide variety of body tissues, including cerebral spinal fluid, semen, pulmonary excretions, placenta, and occasionally urine. Most laboratory-associated cases occur in research facilities and involve exposures to *Brucella* organisms grown in large quantities or exposure to placental tissues containing *Brucella*. Cases have occurred in clinical laboratory settings from sniffing bacteriological cultures[29] or working on open bench tops.[30] Aerosols from, or direct skin contact with, cultures or with infectious clinical specimens from animals (e.g. blood, body fluids, tissues) are commonly implicated in human infections. Aerosols generated during laboratory procedures have caused multiple cases per exposure.[30,31] Mouth pipetting, accidental parenteral inoculations, and sprays into eyes, nose and mouth result in infection. The infectious dose of *Brucella* is 10-100 organisms by aerosol route and subcutaneous route in laboratory animals.[32,33]

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for routine clinical specimens of human or animal origin. Products of conception containing or believed to contain pathogenic *Brucella* should be handled with BSL-3 practices due to the high concentration of organisms per gram of tissue. BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended, for all manipulations of cultures of pathogenic *Brucella* spp. listed in this summary, and for experimental animal studies.

SPECIAL ISSUES

Vaccines Human *Brucella* vaccines have been developed and tested in other countries with limited success. A human vaccine is not available in the United States.[34]

Select Agent *Brucella suis* is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Burkholderia mallei

Burkholderia mallei (formerly *Pseudomonas mallei)* is a non-motile gram-negative rod associated with glanders, a rare disease of equine species and humans.

While endemic foci of infection exist in some areas of the world, glanders due to natural infection is extremely rare in the United States.

Occupational Infections

Glanders occurs almost exclusively among individuals who work with equine species and/or handle *B. mallei* cultures in the laboratory. *B. mallei* can be very infectious in the laboratory setting. The only reported case of human glanders in the United States over the past 50 years resulted from a laboratory exposure.[35] Modes of transmission may include inhalation and/or mucocutaneous exposure.

Natural Modes of Infection

Glanders is a highly communicable disease of horses, goats, and donkeys. Zoonotic transmission occurs to humans, but person-to-person transmission is rare. Clinical glanders no longer occurs in the Western Hemisphere or in most other areas of the world, although enzootic foci are thought to exist in Asia and the eastern Mediterranean.[36] Clinical infections in humans are characterized by tissue abscesses and tend to be very serious.

LABORATORY SAFETY

B. mallei can be very hazardous in a laboratory setting. In a pre-biosafety era report, one-half of the workers in a *B. mallei* research laboratory were infected within a year of working with the organism.[37] Laboratory-acquired infections have resulted from aerosol and cutaneous exposure[37,38] Laboratory infections usually are caused by exposure to bacterial cultures rather than to clinical specimens. Workers should take precautions to avoid exposure to aerosols from bacterial cultures, and to tissues and purulent drainage from victims of this disease.

Containment Recommendations

Primary isolations from patient fluids or tissues may be performed with BSL-2 practices, containment equipment, and facilities in a BSC. Procedures must be performed under BSL-3 containment whenever infectious aerosols or droplets are generated, such as during centrifugation or handling infected animals, or when large quantities of the agent are produced. Procedures conducted outside of a BSC (centrifugation, animal manipulation, etc.) that generate infectious aerosols require respiratory protection. Sealed cups should be used with all centrifuges and these should be opened only inside a BSC. Gloves should be worn when working with potentially infectious material or animals. Animal work with *B. mallei* should be done with ABSL-3 practices, containment equipment, and facilities.

SPECIAL ISSUES

Select Agent *B. mallei* is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Burkholderia pseudomallei

Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*) is a motile gram-negative, oxidase-positive rod that is found in soil and water environments of equatorial regions, including Southeast Asia, Northern Australia, Central America and South America. This organism is the causative agent of melioidosis, an unusual bacterial disease characterized by abscesses in tissues and organs. Victims of the disease frequently exhibit recrudescence months or years after the initial infection.

Occupational Infections

Melioidosis is generally considered to be a disease associated with agriculture; however, *B. pseudomallei* can be hazardous for laboratory workers. There are two reports of melioidosis in laboratory workers who were infected by aerosols or via skin exposure.[39,40] Laboratory workers with diabetes are at increased risk of contracting melioidosis.[41]

Natural Modes of Infection

While primarily a disease found in Southeast Asia and Northern Australia, melioidosis can occasionally be found in the Americas.[42] Natural modes of transmission include the exposure of mucous membranes or damaged skin to soil or water containing the organism, the aspiration or ingestion of contaminated water, or the inhalation of dust from contaminated soil. In endemic areas 5-20% of agricultural workers have antibody titers to *B. pseudomallei*, in the absence of overt disease.[43]

LABORATORY SAFETY

B. pseudomallei can cause a systemic disease in human patients. Infected tissues and purulent drainage from cutaneous or tissue abscesses can be sources of infection. Blood and sputum also are potential sources of infection.

Containment Recommendations

Work with clinical specimens from patients suspected of having melioidosis and of *B. pseudomallei* cultures may be performed with BSL-2 practices, containment equipment, and facilities. Work should be done in a BSC. Gloves always should be worn when manipulating the microorganism. In cases where infectious aerosols or droplets

could be produced, or where production quantities of the organism are generated, these procedures should be confined to BSL-3 facilities with all pertinent primary containment against escape of aerosols. Respiratory protection must be used if the microorganism is manipulated outside of a BSC, such as during centrifugation or handling infected animals. Sealed cups should be used in all centrifuges and these should be opened only in a BSC. Animal studies with this agent should be done at ABSL-3.

SPECIAL ISSUES

Select Agent *B. pseudomallei* is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Campylobacter (C. jejuni subsp. jejuni, C. coli, C. fetus subsp. fetus, C. upsaliensis)

Campylobacters are curved, S-shaped, or spiral rods associated with gastrointestinal infections (primarily *C. jejuni* subsp. *jejuni* and *C. coli*), bacteremia, and sepsis (primarily *C. fetus* subsp. *fetus* and *C. upsaliensis*). Organisms are isolated from stool specimens through the use of selective media, reduced oxygen tension, and elevated incubation temperature (43°C).

Occupational Infections

These organisms rarely cause LAI, although laboratory-associated cases have been documented.[44-47] Experimentally infected animals also are a potential source of infection.[48]

Natural Modes of Infection

Numerous domestic and wild animals, including poultry, pets, farm animals, laboratory animals, and wild birds are known reservoirs and are a potential source of infection for laboratory and animal care personnel. While the infective dose is not firmly established, ingestion of as few as 500-800 organisms has caused symptomatic infection.[49-51] Natural transmission usually occurs from ingestion of organisms in contaminated food or water and from direct contact with infected pets, farm animals, or infants.[52]

LABORATORY SAFETY

Pathogenic *C. sp.* may occur in fecal specimens in large numbers. *C. fetus* subsp. *fetus* may also be present in blood, exudates from abscesses, tissues, and sputa. The primary laboratory hazards are ingestion and parenteral inoculation of *C. jejuni*. The significance of aerosol exposure is not known.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Chlamydia psittaci (Chlamydophila psittaci), C. trachomatis, C. pneumoniae (Chlamydophila pneumoniae)

Chlamydia psittaci, *C. pneumoniae* (sometimes called *Chlamydophila psittaci* and *Chlamydophila pneumoniae*) and *C. trachomatis* are the three species of *Chlamydia* known to infect humans. Chlamydiae are nonmotile, gram-negative bacterial pathogens with obligate intracellular life cycles. These three species of *Chlamydia* vary in host spectrum, pathogenicity, and in the clinical spectrum of disease. *C. psittaci* is a zoonotic agent that commonly infects psittacine birds and is highly pathogenic for humans. *C. trachomatis* is historically considered an exclusively human pathogen and is the most commonly reported bacterial infection in the United States. *C. pneumoniae* is considered the least pathogenic species, often resulting in subclinical or asymptomatic infections in both animals and humans.

Occupational Infections

Chlamydial infections caused by *C. psittaci* and *C. trachomatis* lymphogranuloma venereum (LGV) strains were at one time among the most commonly reported laboratory-associated bacterial infections.[26] In cases reported before 1955,[4] the majority of infections were psittacosis, and these had the highest case fatality rate of laboratory-acquired infectious agents. The major sources of laboratory-associated psittacosis are contact with and exposure to infectious aerosols in the handling, care, or necropsy of naturally or experimentally infected birds. Infected mice and eggs also are important sources of *C. psittaci*. Most reports of laboratory-acquired infections with *C. trachomatis* attribute the infection to inhalation of large quantities of aerosolized organisms during purification or sonification procedures. Early reports commonly attributed infections to exposure to aerosols formed during nasal inoculation of mice or inoculation of egg yolk

sacs and harvest of chlamydial elementary bodies. Infections are associated with fever, chills, malaise, and headache; a dry cough is also associated with *C. psittaci* infection. Some workers exposed to *C. trachomatis* have developed conditions including mediastinal and supraclavicular lymphadenitis, pneumonitis, conjunctivitis, and keratitis.[53] Seroconversion to chlamydial antigens is common and often striking although early antibiotic treatment may prevent an antibody response.

Laboratory-associated infections with *C. pneumoniae* have been reported.[54] Exposed workers were asymptomatic and infection was diagnosed by serology. The route of infection was attributed to inhalation of droplet aerosols created during procedures associated with culture and harvest of the agent from cell culture.

With all species of *Chlamydia*, mucosal tissues in the eyes, nose, and respiratory tract are most often affected by occupational exposures that can lead to infection.

Natural Modes of Infection

C. psittaci is the cause of psittacosis, a respiratory infection that can lead to severe pneumonia requiring intensive care support and possible death. Sequelae include endocarditis, hepatitis, and neurologic complications. Natural infections are acquired by inhaling dried secretions from infected birds. Psittacine birds commonly kept as pets (parrots, parakeets, cockatiels, etc.) and poultry are most frequently involved in transmission. *C. trachomatis* can cause a spectrum of clinical manifestations including genital tract infections, inclusion conjunctivitis, trachoma, pneumonia in infants, and LGV. The LGV strains cause more severe and systemic disease than do genital strains. *C. trachomatis* genital tract infections are sexually transmitted and ocular infections (trachoma) are transmitted by exposure to secretions from infected persons through contact or fomite transmission. *C. pneumoniae* is a common cause of respiratory infection; up to 50% of adults have serologic evidence of previous exposure. Infections with *C. pneumoniae* are transmitted by droplet aerosolization and are most often mild or asymptomatic, although there is a body of evidence associating this agent with chronic diseases such as atherosclerosis and asthma.

LABORATORY SAFETY

C. psittaci may be present in the tissues, feces, nasal secretions and blood of infected birds, and in blood, sputum, and tissues of infected humans. *C. trachomatis* may be present in genital, bubo, and conjunctival fluids of infected humans. Exposure to infectious aerosols and droplets, created during the handling of infected birds and tissues, are the primary hazards to laboratory personnel working with *C. psittaci*. The primary laboratory hazards of *C. trachomatis* and *C. pneumoniae* are accidental parenteral inoculation and direct and indirect exposure of mucous membranes of the eyes, nose, and mouth to genital, bubo, or conjunctival fluids, cell culture materials, and fluids from infected cell cultures or eggs. Infectious aerosols, including those that may be created as a result of centrifuge malfunctions, also pose a risk for infection.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for personnel working with clinical specimens and cultures or other materials known or suspected to contain the ocular or genital serovars (A through K) of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are recommended for activities involving the necropsy of infected birds and the diagnostic examination of tissues or cultures known to contain or be potentially infected with *C. psittaci* strains of avian origin. Wetting the feathers of infected birds with a detergent-disinfectant prior to necropsy can appreciably reduce the risk of aerosols of infected feces and nasal secretions on the feathers and external surfaces of the bird. Activities involving non-avian strains of *C. psittaci* may be performed in a BSL-2 facility as long as BSL-3 practices are followed, including but not limited to the use of primary containment equipment such as BSCs. ABSL-3 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds.

BSL-3 practices and containment equipment are recommended for activities involving work with culture specimens or clinical known to contain or be potentially infected with the LGV serovars (L_1 through L_3) of *C. trachomatis*. Laboratory work with the LGV serovars of *C. trachomatis* can be conducted in a BSL-2 facility as long as BSL-3 practices are followed when handling potentially infectious materials, including but not limited to use of primary containment equipment such as BSCs.

Gloves are recommended for the necropsy of birds and mice, the opening of inoculated eggs, and when there is the likelihood of direct skin contact with infected tissues, bubo fluids, and other clinical materials.

ABSL-2 practices, containment equipment, and facilities are recommended for activities with animals that have been experimentally infected with genital serovars of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are indicated for activities involving any of these species with high potential for droplet or aerosol production and for activities involving large quantities or concentrations of infectious materials.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Clostridium botulinum

Clostridium botulinum, and rare strains of *C. baratii* and *C. butyricum*, are anaerobic spore-forming species that cause botulism, a life-threatening food-borne illness. The pathogenicity of these organisms results from the production of botulinum toxin, one of the most highly potent neurotoxins currently recognized. Purified botulinum neurotoxin is a 150 kDa protein that acts selectively on peripheral cholinergic nerve endings to block neurotransmitter release.[55] The principal site of action is the neuromuscular junction, where blockade of transmission produces muscle weakness or paralysis. The toxin also acts on autonomic nerve endings where blockade of transmission can produce a variety of adverse effects. The toxin may also contain associated proteins that may increase its size to as high as 900 kDA.

Occupational Infections

There has been only one report of botulism associated with handling of the toxin in a laboratory setting.[56] However, concerns about potential use of the toxin as an agent of bioterrorism or biological warfare have led to increased handling of the substance by investigators studying mechanism of action and/or developing countermeasures to poisoning.[57]

Natural Modes of Infection

Botulinum toxin occurs in seven different serotypes (A to G), but almost all naturally-occurring human illness is due to serotypes A, B, E, and F.[58] Poisoning is normally due to ingestion of toxin; however, animal studies show a significant inhalation risk. Using appropriate personal protective equipment should prevent potential exposure through mucus membranes. Symptoms and even death are possible by accidental injection of botulinum toxin. Although rare, botulism also can be caused by puncture wounds in which there is local infection and *in situ* production of toxin. Risk to toxin exposure is dependent on both route of exposure and toxin molecular weight size. Ingestion of spores by adults with a compromised gastrointestinal tract (GI), such as following GI surgery or long-term administration of antibiotics, may increase risk for intestinal infection and *in situ* production of toxin. See the *C. botulinum* Toxin Agent Summary Statement and Appendix I for additional information.

LABORATORY SAFETY

C. botulinum or its toxin may be present in a variety of food products, clinical materials (serum, feces) and environmental samples (soil, surface water).[59] In addition, bacterial cultures may produce very high levels of toxin.[60] In healthy adults, it is typically the toxin and not the organism that causes disease.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin[61] including the handling of potentially contaminated food. Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N)

readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Autoclaving of contaminated materials also is appropriate.

BSL-3 practices, containment equipment, and facilities are required for activities with a high potential for aerosol or droplet production, and for those involving large quantities of the organism or of the toxin. ABSL-2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.

SPECIAL ISSUES

Vaccines A pentavalent (A, B, C, D and E) botulinum toxoid vaccine (PBT) is available through the CDC as an IND. Vaccination is recommended for all personnel working in direct contact with cultures of *C. botulinum* or stock solutions of botulinum neurotoxin (BoNT). Due to a recent decline in immunogenicity of available PBT stocks for some toxin serotypes, the immunization schedule for the PBT recently has been modified to require injections at 0, 2, 12, and 24 weeks followed by a booster at 12 months and annual boosters thereafter.

Post-Exposure Treatment An equine antitoxin product is available for treatment of patients with symptoms consistent with botulism. However, due to the risks inherent in equine products, treatment is not provided as a result of exposure unless botulism symptoms are present.

Select Agent *C. botulinum* toxin is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Clostridium tetani and Tetanus toxin

Clostridium tetani is an anaerobic endospore-forming gram-positive rod found in the soil and an intestinal tract commensal. It produces a potent neurotoxin, tetanospasmin, that causes tetanus, an acute neurologic condition characterized by painful muscular contractions. Tetanospasmin is an exceedingly potent, high molecular weight protein toxin, consisting of a heavy chain (100kD) subunit that binds the toxin to receptors on neuronal cells and a light chain (50kD) subunit that blocks the release of inhibitory neural transmitter molecules within the central nervous system. The incidence of tetanus in the United States has declined steadily since the introduction of tetanus toxoid vaccines in the 1940's.[62]

Occupational Infections

Although the risk of infection to laboratory personnel is low, there have been five incidents of laboratory personnel exposure recorded.[4]

Natural Modes of Infection

Contamination of wounds by soil is the usual mechanism of transmission for tetanus. Of the 130 cases of tetanus reported to CDC from 1998 through 2000, acute injury (puncture, laceration, abrasion) was the most frequent predisposing condition. Elevated incidence rates also were observed for persons aged over 60 years, diabetics, and intravenous drug users.[63] When introduced into a suitable anaerobic or microaerophilic environment, *C. tetani* spores germinate and produce tetanospasmin. The incubation period ranges from 3 to 21 days. The observed symptoms are primarily associated with the presence of the toxin. Wound cultures are not generally useful for diagnosing tetanus.[64]

LABORATORY SAFETY

The organism may be found in soil, intestinal, or fecal samples. Accidental parenteral inoculation of the toxin is the primary hazard to laboratory personnel. Because it is uncertain if tetanus toxin can be absorbed through mucous membranes, the hazards associated with aerosols and droplets remain unclear.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities involving the manipulation of cultures or toxin. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies.

SPECIAL ISSUES

Vaccines The vaccination status of workers should be considered in a risk assessment for workers with this organism and/or toxin. While the risk of laboratory-associated tetanus is low, the administration of an adult diphtheria-tetanus toxoid at 10-year intervals further reduces the risk to laboratory and animal care personnel of toxin exposures and wound contamination, and is therefore highly recommended.[62] The reader is advised to consult the current recommendations of the ACIP. [65]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Corynebacterium diphtheriae

Cornebacterium diphtheriae is a pleomorphic gram-positive rod that is isolated from the nasopharynx and skin of humans. The organism is easily grown in the

laboratory on media containing 5% sheep blood. *C. diphtheriae* produces a potent exotoxin and is the causative agent of diphtheria, one of the most wide-spread bacterial diseases in the pre-vaccine era.

Occupational Infections

Laboratory-associated infections with *C. diphtheriae* have been documented, but laboratory animal-associated infections have not been reported.[4,66] Inhalation, accidental parenteral inoculation, and ingestion are the primary laboratory hazards.

Natural Modes of Infection

The agent may be present in exudates or secretions of the nose, throat (tonsil), pharynx, larynx, wounds, in blood, and on the skin. Travel to endemic areas or close contact with persons who have returned recently from such areas, increases risk.[67] Transmission usually occurs via direct contact with patients or carriers, and more rarely, with articles contaminated with secretions from infected people. Naturally occurring diphtheria is characterized by the development of grayish-white membranous lesions involving the tonsils, pharynx, larynx, or nasal mucosa. Systemic sequelae are associated with the production of diphtheria toxin. An effective vaccine has been developed for diphtheria and this disease has become a rarity in countries with vaccination programs.

LABORATORY SAFETY

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. ABSL-2 facilities are recommended for studies utilizing infected laboratory animals.

SPECIAL ISSUES

Vaccines A licensed vaccine is available. The reader is advised to consult the current recommendations of the ACIP. [67] While the risk of laboratory-associated diphtheria is low, the administration of an adult diphtheria-tetanus toxoid at 10-year intervals may further reduce the risk of illness to laboratory and animal care personnel.[67]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Francisella tularensis

Francisella tularensis is a small gram-negative coccobacillus that is carried in numerous animal species, especially rabbits, and is the causal agent of tularemia (Rabbit

fever, Deer fly fever, Ohara disease, or Francis disease) in humans. *F. tularensis* can be divided into three subspecies, *F. tularensis* (Type A), *F. holarctica* (Type B) and *F. novicida*, based on virulence testing, 16S sequence, biochemical reactions and epidemiologic features. Type A and Type B strains are highly infectious, requiring only 10-50 organisms to cause disease. Subspecies *F. novicida* is infrequently identified as the cause of human disease. Person-to-person transmission of tularemia has not been documented. The incubation period varies with the virulence of the strain, dose and route of introduction but ranges from 1-14 days with most cases exhibiting symptoms in 3-5 days.[68]

Occupational Infections

Tularemia has been a commonly reported laboratory-associated bacterial infection.[4] Most cases have occurred at facilities involved in tularemia research; however, cases have been reported in diagnostic laboratories as well. Occasional cases were linked to work with naturally or experimentally infected animals or their ectoparasites.

Natural Modes of Infection

Tick bites, handling or ingesting infectious animal tissues or fluids, ingestion of contaminated water or food and inhalation of infective aerosols are the primary transmission modes in nature. Occasionally infections have occurred from bites or scratches by carnivores with contaminated mouth parts or claws.

LABORATORY SAFETY

The agent may be present in lesion exudates, respiratory secretions, cerebrospinal fluid (CSF), blood, urine, tissues from infected animals, fluids from infected animals, and fluids from infected arthropods. Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, and exposure to aerosols and infectious droplets has resulted in infection. Infection has been more commonly associated with cultures than with clinical materials and infected animals.[69]

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities involving clinical materials of human or animal origin suspected or known to contain *F. tularensis*. Laboratory personnel should be informed of the possibility of tularemia as a differential diagnosis when samples are submitted for diagnostic tests. BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies and for experimental animal studies. Preparatory work on cultures or contaminated materials for automated identification systems should be performed at BSL-3. Characterized strains of reduced virulence such as *F. tularensis* Type B (strain LVS) and *F. tularensis* subsp *novicida* (strain U112) can be manipulated in BSL-2. Manipulation of reduced virulence strains at high concentrations should be conducted using BSL-3 practices.

SPECIAL ISSUES

Select Agent *F. tularensis* is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Helicobacter species

Helicobacters are spiral or curved gram-negative rods isolated from gastrointestinal and hepatobiliary tracts of mammals and birds. There are currently 20 recognized species, including at least nine that have been isolated from humans. Since its discovery in 1982, *Helicobacter pylori* has received increasing attention as an agent of gastritis.[70] The main habitat of *H. pylori* is the human gastric mucosa. Other *Helicobacter* spp. (*H. cinaedi, H. canadensis, H. canis, H. pullorum,* and *H. fennelliae*) may cause asymptomatic infection as well as proctitis, proctocolitis, enteritis and extraintestinal infections in humans.[71,72] *H. cinaedi* has been isolated from dogs, cats and Syrian hamsters.

Occupational Infections

Both experimental and accidental LAI with *H. pylori* have been reported.[73,74] Ingestion is the primary known laboratory hazard. The importance of aerosol exposures is unknown.

Natural Modes of Infection

Chronic gastritis and duodenal ulcers are associated with *H. pylori* infection. Epidemiologic associations have also been made with gastric adenocarcinoma. Human infection with *H. pylori* may be long in duration with few or no symptoms, or may present as an acute gastric illness. Transmission, while incompletely understood, is thought to be by the fecal-oral or oral-oral route.

LABORATORY SAFETY

H. pylori may be present in gastric and oral secretions and stool.[75] The enterohepatic helicobacters (e.g., *H. canadensis, H. canis, H, cinaedi, H. fennelliae, H. pullorum,* and *H. winghamensis*) may be isolated from stool specimens, rectal swabs, and blood cultures.[72] Protocols involving homogenization or vortexing of gastric specimens have been described for the isolation of *H. pylori*.[76] Containment of potential aerosols or droplets should be incorporated in these procedures.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially contain the agents. ABSL-2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Legionella pneumophila and other Legionella-like Agents

Legionella are small, faintly staining gram-negative bacteria. They are obligately aerobic, slow-growing, nonfermentative organisms that have a unique requirement for L-cysteine and iron salts for *in vitro* growth. Legionellae are readily found in natural aquatic bodies and some species (*L. longbeachae*) have been recovered from soil.[77,78] They are able to colonize hot-water tanks at a temperature range from 40 to 50°C. There are currently 48 known *Legionella* species, 20 of which have been associated with human disease. *L. pneumophila* is the species most frequently encountered in human infections.[79-81]

Occupational Infections

Although laboratory-associated cases of legionellosis have not been reported in the literature, at least one case, due to presumed aerosol or droplet exposure during animal challenge studies with *L. pneumophila*, has been recorded.[82] Experimental infections have been produced in guinea pigs, mice, rats, embryonated chicken eggs, and human or animal cell lines.[83] A fatal case of pneumonia due to *L. pneumophila* was diagnosed in a calf, but only 1.7% (2/112) of the other cattle in the herd had serological evidence of exposure to *Legionella*.[84] The disease was linked to exposure to a hot water system colonized with *Legionella*. Animal-to-animal transmission has not been demonstrated.

Natural Modes of Infection

Legionella is commonly found in environmental sources, typically in man-made warm water systems. The mode of transmission from these reservoirs is aerosolization, aspiration or direct inoculation into the airway.[85] Direct person-to-person transmission does not occur. The spectrum of illness caused by *Legionella* species ranges from a mild, self-limited flu-like illness (Pontiac fever) to a disseminated and often fatal disease characterized by pneumonia and respiratory failure (Legionnaires disease). Although

rare, *Legionella* has been implicated in cases of sinusitis, cellulitis, pericarditis, and endocarditis.[86] Legionellosis may be either community-acquired or nosocomial. Risk factors include smoking, chronic lung disease, and immunosuppression. Surgery, especially involving transplantation, has been implicated as a risk factor for nosocomial transmission.

LABORATORY SAFETY

The agent may be present in respiratory tract specimens (sputum, pleural fluid, bronchoscopy specimens, lung tissue), and in extrapulmonary sites. A potential hazard may exist for generation of aerosols containing high concentrations of the agent.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of potentially infectious materials, including minimizing the potential for dissemination of the organism from cultures of organisms known to cause disease. ABSL-2 practices, containment equipment and facilities are recommended for activities with experimentally-infected animals. Routine processing of environmental water samples for *Legionella* may be performed with standard BSL-2 practices. For activities likely to produce extensive aerosols and when large quantities of the pathogenic organisms are manipulated, BSL-2 with BSL-3 practices is recommended.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Leptospira

The genus *Leptospira* is composed of spiral-shaped bacteria with hooked ends. Leptospires are ubiquitous in nature, either free-living in fresh water or associated with renal infection in animals. Historically, these organisms have been classified into pathogenic (*L. interrogans*) and saprophytic (*L. biflexa*) groups, but recent studies have identified more than 12 species based on genetic analysis. These organisms also have been characterized serologically, with more than 200 pathogenic and 60 saprophytic serovars identified as of 2003.[87] These organisms are the cause of leptospirosis, a zoonotic disease of worldwide distribution. Growth of leptospires in the laboratory requires specialized media and culture techniques, and cases of leptospirosis are usually diagnosed by serology.

Occupational Infections

Leptospirosis is a well-documented laboratory hazard. Approximately, 70 LAI and 10 deaths have been reported.[4,26] Direct and indirect contact with fluids and tissues of experimentally or naturally infected mammals during handling, care, or necropsy are potential sources of infection.[88-90] It is important to remember that rodents are natural carriers of leptospires. Animals with chronic renal infection shed large numbers of leptospires in the urine continuously or intermittently, for long periods of time. Rarely, infection may be transmitted by bites of infected animals.[88]

Natural Modes of Infection

Human leptospirosis typically results from direct contact with infected animals, contaminated animal products, or contaminated water sources. Common routes of infection include abrasions, cuts in the skin or via the conjunctiva. Higher rates of infection observed in agricultural workers and other occupations associated with animal contact.

LABORATORY SAFETY

The organism may be present in urine, blood, and tissues of infected animals and humans. Ingestion, accidental parenteral inoculation, and direct and indirect contact of skin or mucous membranes, particularly the conjunctiva, with cultures or infected tissues or body fluids are the primary laboratory hazards. The importance of aerosol exposure is not known.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infective tissues, body fluids, and cultures. The housing and manipulation of infected animals should be performed at ABSL-2. Gloves should be worn to handle and necropsy infected animals and to handle infectious materials and cultures in the laboratory.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Listeria monocytogenes

Listeria monocytogenes is a gram-positive, non-spore-forming, aerobic bacillus; that is weakly beta-hemolytic on sheep blood agar and catalase-positive.[91] The organism has been isolated from soil, animal feed (silage) and a wide range of human foods and food processing environments. It may also be isolated from symptomatic/asymptomatic

animals (particularly ruminants) and humans.[91,92] This organism is the causative agent of listeriosis, a food-borne disease of humans and animals.

Occupational Infections

Cutaneous listeriosis, characterized by pustular or papular lesions on the arms and hands, has been described in veterinarians and farmers.[93] Asymptomatic carriage has been reported in laboratorians.[94]

Natural Modes of Infection

Most human cases of listeriosis result from eating contaminated foods, notably soft cheeses, ready-to-eat meat products (hot dogs, luncheon meats), paté and smoked fish/seafood.[95] Listeriosis can present in healthy adults with symptoms of fever and gastroenteritis, pregnant women and their fetuses, newborns, and persons with impaired immune function are at greatest risk of developing severe infections including sepsis, meningitis, and fetal demise. In pregnant women, *Listeria monocytogenes* infections occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus.[92]

LABORATORY SAFETY

Listeria monocytogenes may be found in feces, CSF, and blood, as well as numerous food and environmental samples.[91,92,96,97] Naturally or experimentally infected animals are a source of exposure to laboratory workers, animal care personnel and other animals. While ingestion is the most common route of exposure, *Listeria* can also cause eye and skin infections following direct contact with the organism.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended when working with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn while handling infected or potentially infected materials. ABSL-2 practices, containment equipment and facilities are recommended for activities involving experimentally or naturally infected animals. Due to potential risks to the fetus, pregnant women should be advised of the risk of exposure to *L. monocytogenes*.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Mycobacterium leprae

Mycobacterium leprae is the causative agent of leprosy (Hansen disease). The organism has not been cultivated in laboratory medium but can be maintained in a metabolically active state for some period. Organisms are recovered from infected tissue and can be propagated in laboratory animals, specifically armadillos and the footpads of mice. The infectious dose in humans is unknown. Although naturally occurring leprosy or leprosy-like diseases have been reported in armadillos[98] and in NHP,[99,100] humans are the only known important reservoir of this disease.

Occupational Infections

There are no cases reported as a result of working in a laboratory with biopsy or other clinical materials of human or animal origin. However, inadvertent human-to-human transmissions following an accidental needle stick by a surgeon and after use of a presumably contaminated tattoo needle were reported prior to 1950.[101,102]

Natural Modes on Infection

Leprosy is transmitted from person-to-person following prolonged exposure, presumably via contact with secretions from infected individuals.

LABORATORY SAFETY

The infectious agent may be present in tissues and exudates from lesions of infected humans and experimentally or naturally infected animals. Direct contact of the skin and mucous membranes with infectious materials and accidental parenteral inoculation are the primary laboratory hazards associated with handling infectious clinical materials. See Appendix B for appropriate tuberculocidal disinfectant.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious materials from humans and animals. Extraordinary care should be taken to avoid accidental parenteral inoculation with contaminated sharp instruments. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies utilizing rodents, armadillos, and NHP, because coughing with dissemination of infectious droplets does not occur in these species.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Mycobacterium tuberculosis complex

The *Mycobacterium tuberculosis* complex includes *M. tuberculosis, M. bovis, M. africanum,* and *M. microti* that cause tuberculosis in humans, and more recently recognized *M. caprae* and *M. pinnipedii* that have been isolated from animals. *M. tuberculosis* grows slowly, requiring three weeks for formation of colonies on solid media. The organism has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents and allows them to stain acid-fast.

Occupational Infections

M. tuberculosis and *M. bovis* infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities.[4,26,103-105] The incidence of tuberculosis in laboratory personnel working with *M. tuberculosis* has been reported to be three times higher than that of those not working with the agent.[106] Naturally or experimentally infected NHP are a proven source of human infection.[107] Experimentally infected guinea pigs or mice do not pose the same hazard because droplet nuclei are not produced by coughing in these species; however, litter from infected animal cages may become contaminated and serve as a source of infectious aerosols.

Natural Mode of Infection

M. tuberculosis is the etiologic agent of tuberculosis, a leading cause of morbidity and mortality worldwide. Persons infected with *M. tuberculosis* can develop active disease within months of infection or can remain latently infected and develop disease later in life. The primary focus of infection is the lungs, but most other organs can be involved. HIV infection is a serious risk factor for development of active disease. Tuberculosis is spread by infectious aerosols produced by coughing. *M. bovis* is primarily found in animals but also can produce tuberculosis in humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and milk products, by handling of infected carcasses, and by inhalation. Human-to-human transmission via aerosols also is possible.

LABORATORY SAFETY

Tubercle bacilli may be present in sputum, gastric lavage fluids, CSF, urine, and in a variety of tissues. Exposure to laboratory-generated aerosols is the most important hazard encountered. Tubercle bacilli may survive in heat-fixed smears[108] and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low infective dose of *M. tuberculosis* (i.e., ID50 <10 bacilli), sputa and other clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. Accidental needle-sticks are also a recognized hazard.

Containment Recommendations

BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. Liquifaction and concentration of sputa for acid-fast staining may be conducted safely on the open bench by first treating the specimen in a BSC with an equal volume of 5% sodium hypochlorite solution (undiluted household bleach) and waiting 15 minutes before processing.[109,110]

BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP. Animal studies using guinea pigs or mice can be conducted at ABSL-2.[111] BSL-3 practices should include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols. Disinfectants proven to be tuberculocidal should be used. See Appendix B for additional information.

Manipulation of small quantities of the attenuated vaccine strain *M. bovis* Bacillus Calmette-Guérin (BCG) can be performed at BSL-2 in laboratories that do not culture *M. tuberculosis* and do not have BSL-3 facilities. However, considerable care must be exercised to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

SPECIAL ISSUES

Surveillance Annual or semi-annual skin testing with purified protein derivative (PPD) of previously skin-test-negative personnel can be used as a surveillance procedure.

Vaccines The attenuated live BCG, is available and used in other countries but is not used in the United States for immunization.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Mycobacterium spp. other than M. tuberculosis complex and M. leprae

More than 100 species of mycobacteria are recognized. These include both slowly growing and rapidly growing species. In the past, mycobacterial isolates that were not

identified as *M. tuberculosis* complex were often called atypical mycobacteria, but these are now more commonly referred to as nontuberculous mycobacteria or mycobacteria other than tuberculosis. Many of the species are common environmental organisms, and approximately 25 of them are associated with infections in humans. A number of additional species are associated with infections in immunocompromised persons, especially HIV-infected individuals. All of these species are considered opportunistic pathogens in humans and none are considered communicable. Mycobacteria are frequently isolated from clinical samples but may not be associated with disease. The most common types of infections and causes are:

1. pulmonary disease with a clinical presentation resembling tuberculosis caused by *M. kansasii*, *M. avium*, and *M. intracellulare;*

2. lymphadenitis associated with *M. avium* and *M. scrofulaceum;*

3. disseminated infections in immunocompromised individuals caused by *M. avium*;

4. skin ulcers and soft tissue wound infections including Buruli ulcer caused by *M. ulcerans*, swimming pool granuloma caused by *M. marinum* associated with exposure to organisms in fresh and salt water and fish tanks, and tissue infections resulting from trauma, surgical procedures, or injection of contaminated materials caused by *M. fortuitum*, *M. chelonei*, and *M. abscesens*.

Occupational Infections

Laboratory-acquired infections with *Mycobacterium* spp. other than *M. tuberculosis* complex have not been reported.

Natural Modes of Infection

Person-to-person transmission has not been demonstrated. Presumably, pulmonary infections are the result of inhalation of aerosolized bacilli, most likely from the surface of contaminated water. Mycobacteria are widely distributed in the environment and in animals. They are also common in potable water supplies, perhaps as the result of the formation of biofilms. The source of *M. avium* infections in immunocompromised persons has not been established.

LABORATORY SAFETY

Various species of mycobacteria may be present in sputa, exudates from lesions, tissues, and in environmental samples. Direct contact of skin or mucous membranes with infectious materials, ingestion, and accidental parenteral inoculation are the primary laboratory hazards associated with clinical materials and cultures. Aerosols created

during the manipulation of broth cultures or tissue homogenates of these organisms also pose a potential infection hazard.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacteria* spp. other than *M. tuberculosis* complex. Clinical specimens may also contain *M. tuberculosis* and care must be exercised to ensure the correct identification of cultures. Special caution should be exercised in handling *M. ulcerans* to avoid skin exposure. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Neisseria gonorrhoeae

Neisseria gonorrhoeae is a gram-negative, oxidase-positive diplococcus associated with gonorrhea, a sexually transmitted disease of humans. The organism may be isolated from clinical specimens and cultivated in the laboratory using specialized growth media.[112]

Occupational Infections

Laboratory-associated gonococcal infections have been reported in the United States and elsewhere.[113-116] These infections have presented as conjunctivitis, with either direct finger-to-eye contact or exposure to splashes of either liquid cultures or contaminated solutions proposed as the most likely means of transmission.

Natural Modes of Infection

Gonorrhea is a sexually transmitted disease of worldwide importance. The 2004 rate of reported infections for this disease in the United States was 112 per 100,000 population.[117] The natural mode of infection is through direct contact with exudates from mucous membranes of infected individuals. This usually occurs by sexual activity, although newborns may also become infected during birth.[112]

LABORATORY SAFETY

The agent may be present in conjunctival, urethral and cervical exudates, synovial fluid, urine, feces, and CSF. Accidental parenteral inoculation and direct or indirect contact of mucous membranes with infectious clinical materials are known primary laboratory hazards. Laboratory-acquired illness due to aerosol transmission has not been documented.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical materials or cultures. Gloves should be worn when handling infected laboratory animals and when there is the likelihood of direct skin contact with infectious materials. Additional primary containment and personnel precautions such as those described for BSL- 3 may be indicated when there is high risk of aerosol or droplet production, and for activities involving production quantities or high concentrations of infectious materials. Animal studies may be performed at ABSL-2.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Neisseria meningitidis

Neisseria meningitidis is a gram-negative coccus responsible for serious acute meningitis and septicemia in humans. Virulence is associated with the expression of a polysaccharide capsule. Thirteen different capsular serotypes have been identified, with types A, B, C, Y, and W135 associated with the highest incidence of disease. The handling of invasive *N. meningitidis* isolates from blood or CSF represents an increased risk to microbiologists.[118,119]

Occupational Infections

Recent studies of LAI and exposures have indicated that manipulating suspensions of *N. meningitidis* outside a BSC is associated with a high risk for contracting meningococcal disease.[119] Investigations of potential laboratory-acquired cases of meningococcal diseases in the United States showed a many-fold higher attack rate for microbiologists compared to that of the United States general population age 30-59 years, and a case fatality rate of 50%, substantially higher than the 12-15% associated with disease among the general population. Almost all the microbiologists had manipulated sterile site isolates on an open laboratory bench.[120] While isolates obtained from respiratory sources are generally less pathogenic and consequently represent lower risk for microbiologists, rigorous protection from droplets or aerosols is mandated when

microbiological procedures are performed on all *N. meningitidis* isolates, especially on those from sterile sites.

Natural Modes of Infection

The human upper respiratory tract is the natural reservoir for *N. meningitidis.* Invasion of organisms from the respiratory mucosa into the circulatory system causes infection that can range in severity from subclinical to fulminant fatal disease. Transmission is person-to-person and is usually mediated by direct contact with respiratory droplets from infected individuals.

LABORATORY SAFETY

N. meningitidis may be present in pharyngeal exudates, CSF, blood, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, infectious aerosol and ingestion are the primary hazards to laboratory personnel. Based on the mechanism of natural infection and the risk associated with handling of isolates on an open laboratory bench, exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for infection in the laboratory.

Containment Recommendations

Specimens for *N. meningitidis* analysis and cultures of *N. meningitidis* not associated with invasive disease may be handled in BSL-2 facilities with rigorous application of BSL-2 standard practices, special practices, and safety equipment. All sterile-site isolates of *N. meningitidis* should be manipulated within a BSC. Isolates of unknown source should be treated as sterile-site isolates. If a BSC is unavailable, manipulation of these isolates should be minimized, primarily focused on serogroup identification using phenolized saline solution while wearing laboratory coat, gloves, and safety glasses or full face splash shield. BSL-3 practices and procedures are indicated for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. Animal studies should be performed under ABSL-2 conditions.

SPECIAL ISSUES

Vaccines The quadrivalent meningococcal polysaccharide vaccine, which includes serogroups A, C, Y, and W-135, will decrease but not eliminate the risk of infection, because it is less than 100% effective and does not provide protection against serogroup B, which caused one-half of the laboratory-acquired cases in the United States in 2000.[118,120] Laboratorians who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination.[118,121,122]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Salmonella serotypes, other than S. Typhi

Salmonellae are gram-negative enteric bacteria associated with diarrheal illness humans. They are motile oxidase-negative organisms that are easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation from clinical materials. Recent taxonomic studies have organized this genus into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.[123] *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotypes Typhimuruim and Enteritidis (commonly designated *S.* Typhimurium and *S.* Enteritidis) are the serotypes most frequently encountered in the United States. This summary statement covers all pathogenic serotypes except *S.* Typhi.

Occupational Infections

Salmonellosis is a documented hazard to laboratory personnel.[4,26,124-125] Primary reservoir hosts include a broad spectrum of domestic and wild animals, including birds, mammals, and reptiles, all of which may serve as a source of infection to laboratory personnel. Case reports of laboratory-acquired infections indicate a presentation of symptoms (fever, severe diarrhea, abdominal cramping) similar to those of naturally-acquired infections, although one case also developed erythema nodosum and reactive arthritis.[126,127]

Natural Modes of Infection

Salmonellosis is a foodborne disease of worldwide distribution. An estimated 5 million cases of salmonellosis occur annually in the United States. A wide range of domestic and feral animals (poultry, swine, rodents, cattle, iguanas, turtles, chicks, dogs, cats) may serve as reservoirs for this disease, as well as humans.[128] The most common mode of transmission is by ingestion of food from contaminated animals or contaminated during processing. The disease usually presents as an acute enterocolitis, with an incubation period ranging from 6 to 72 hours.

LABORATORY SAFETY

The agent may be present in feces, blood, urine, and in food, feed, and environmental materials. Ingestion or parenteral inoculation are the primary laboratory hazards. The importance of aerosol exposure is not known. Naturally or experimentally infected animals are a potential source of infection for laboratory and animal care personnel, and for other animals.

Containment Recommendations

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as a BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Salmonella Typhi

Recent taxonomic studies have organized the genus *Salmonella* into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.[123] *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotype Typhi, commonly designated *S.* Typhi, is the causative agent of typhoid fever. *S.* Typhi is a motile gram-negative enteric bacterium that is easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation of this organism from clinical materials.

Occupational Infections

Typhoid fever is a demonstrated hazard to laboratory personnel.[4,129,130] Ingestion and less frequently, parenteral inoculation are the most significant modes of transmission in the laboratory. Secondary transmission to other individuals outside of the laboratory is also a concern.[131] Laboratory-acquired *S.* Typhi infections usually present with symptoms of septicemia, headache, abdominal pain, and high fever.[129]

Natural Modes of Infection

Typhoid fever is a serious, potentially lethal bloodstream infection of worldwide distribution. Humans are the sole reservoir and asymptomatic carriers may occur. The infectious dose is low ($<10^3$ organisms) and the incubation period may vary from one to six weeks, depending upon the dose of the organism. The natural mode of transmission is

by ingestion of food or water contaminated by feces or urine of patients or asymptomatic carriers.[123]

LABORATORY SAFETY

The agent may be present in feces, blood, gallbladder (bile), and urine. Humans are the only known reservoir of infection. Ingestion and parenteral inoculation of the organism represent the primary laboratory hazards. The importance of aerosol exposure is not known.

Containment Recommendations

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as a BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. BSL-3 practices and equipment are recommended for activities likely to produce significant aerosols or for activities involving production quantities of organisms. ABSL-2 facilities, practices and equipment are recommended for activities with experimentally infected animals. ABSL-3 conditions may be considered for protocols involving aerosols.

SPECIAL ISSUES

Vaccines Vaccines for S. Typhi are available and should be considered for personnel regularly working with potentially infectious materials. The reader is advised to consult the current recommendations of the Advisory committee on Immunization Practices (ACIP) published in the CDC Morbidity and Mortality Weekly Report for recommendations for vaccination against S. Typhi.[132]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Shiga toxin (Verocytotoxin)-producing Escherichia coli

Escherichi coli is one of five species in the gram-negative genus *Escherichia*. This organism is a common inhabitant of the bowel flora of healthy humans and other mammals and is one of the most intensively studied prokaryotes. An extensive serotyping

system has been developed for *E. coli* based the O (somatic) and H (flagellar) antigens expressed by these organisms. Certain pathogenic clones of *E. coli* may cause urinary tract infections, bacteremia, meningitis, and diarrheal disease in humans, and these clones are associated with specific serotypes.

The diarrheagenic *E. coli* strains have been characterized into at least four basic pathogenicity groups: Shiga toxin (Verocytotoxin)-producing *E. coli* (a subset of which are referred to as enterohemorrhagic *E. coli*), enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli.* [123] In addition to clinical significance, *E. coli* strains are commonly-used hosts for cloning experiments and other genetic manipulations in the laboratory. This summary statement provides recommendations for safe manipulation of Shiga toxin-producing *E. coli* strains. Procedures for safely handling laboratory derivatives of *E. coli* or other pathotypes of *E. coli* should be based upon a thorough risk assessment.

Occupational Infections

Shiga toxin-producing *E. coli* strains, including strains of serotype O157:H7, are a demonstrated hazard to laboratory personnel.[133-138] The infectious dose is estimated to be low—similar to that reported for *Shigella* spp., 10-100 organisms.[136] Domestic farm animals (particularly bovines) are significant reservoirs of the organisms; however, experimentally infected small animals are also sources of infection in the laboratory.[139] Verocytotoxin-producing *Escherichia coli* have also been in wild birds and rodents in close proximity to farms.[140]

Natural Modes of Infection

Cattle represent the most common natural reservoir of Shiga-toxin producing *E. coli.* Transmission usually occurs by ingestion of contaminated food, including raw milk, fruits, vegetables, and particularly ground beef. Human-to-human transmission has been observed in families, day care centers, and custodial institutions. Water-borne transmission has been reported from outbreaks associated with swimming in a crowded lake and drinking unchlorinated municipal water.[139] In a small proportion of patients (usually children) infected with these organisms, the disease progresses to hemolytic uremic syndrome or death.

LABORATORY SAFETY

Shiga toxin-producing *E. coli* are usually isolated from feces. However, a variety of food specimens contaminated with the organisms including uncooked ground beef, unpasteurized dairy products and contaminated produce may present laboratory hazards. This agent may be found in blood or urine specimens from infected humans or animals. Accidental ingestion is the primary laboratory hazard. The importance of aerosol exposure is not known.

Containment Recommendations

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment or in devices such as a BSC or safety centrifuge cups. Personal protective equipment, such as splash shields, face protection, gowns, and gloves should be used in accordance with a risk assessment. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 practices and facilities are recommended for activities with experimentally or naturally infected animals.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Shigella

The genus *Shigella* is composed of nonmotile gram-negative bacteria in the family Enterobacteriaceae. There are four subgroups that have been historically treated as separate species, even though more recent genetic analysis indicates that they are members of the same species. These include subgroup A (*Shigella dysenteriae*), subgroup B (*S. flexneri*), subgroup C (*S. boydii*), and subgroup D (*S. sonnei*). Members of the genus *Shigella* have been recognized since the late 19th century as causative agents of bacillary dysentery, or shigellosis.[123]

Occupational Infections

Shigellosis is one of the most frequently reported laboratory-acquired infections in the United States.[131,141] A survey of 397 laboratories in the United Kingdom revealed that in 1994-1995, four of nine reported laboratory-acquired infections were caused by *Shigella*.[142] Experimentally infected guinea pigs, other rodents, and NHP are proven sources of laboratory-acquired infection.[143,144]

Natural Modes of Infection

Humans and other large primates are the only natural reservoirs of *Shigella* bacteria. Most transmission is by fecal-oral route; infection also is caused by ingestion of contaminated food or water.[123] Infection with *Shigella dysenteriae* type 1 causes more severe, prolonged, and frequently fatal illness than does infection with other *Shigella*.

Complications of shigellosis include hemolytic uremic syndrome, which is associated with *S. dysenteriae* 1 infection, and Reiter chronic arthritis syndrome, which is associated with *S. flexneri* infection.

LABORATORY SAFETY

The agent may be present in feces and, rarely, in the blood of infected humans or animals. Accidental ingestion and parenteral inoculation of the agent are the primary laboratory hazards. The 50% infectious dose (oral) of *Shigella* for humans is only a few hundred organisms.[143] The importance of aerosol exposure is not known.

Containment Recommendations

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment such as a BSC or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally or naturally infected animals.

SPECIAL ISSUES

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Treponema pallidum

Treponema pallidum is a species of extremely fastidious spirochetes that die readily upon desiccation or exposure to atmospheric levels of oxygen, and have not been cultured continuously in vitro.[145] *T. pallidum* cells have lipid-rich outer membranes and are highly susceptible to disinfection with common alcohols (i.e. 70% isopropanol). This species contains three subspecies including *T. pallidum* ssp. *pallidum* (associated with venereal syphilis), *T. pallidum* ssp. *endemicum* (associated with endemic syphilis), and *T. pallidum* ssp. *pertenue* (associated with Yaws). These organisms are obligate human pathogens.

Occupational Infections

T. pallidum is a documented hazard to laboratory personnel. Pike lists 20 cases of LAI.[4] Syphilis has been transmitted to personnel working with a concentrated suspension of *T. pallidum* obtained from an experimental rabbit orchitis.[146] *T. pallidum* is present in the circulation during primary and secondary syphilis. The ID50 of *T. pallidum* needed to infect rabbits by subcutaneous injection has been reported to be as low as 23 organisms.[147] The concentration of *T. pallidum* in patients' blood during early syphilis, however, has not been determined. No cases of laboratory animal-associated infections are reported; however, rabbit-adapted *T. pallidum* (Nichols strain and possibly others) retains virulence for humans.

Natural Modes of Infection

Humans are the only known natural reservoir of *T. pallidum* and transmission occurs via direct sexual contact (venereal syphilis), direct skin contact (Yaws), or direct mucous contact (endemic syphilis). Venereal syphilis is a sexually transmitted disease that occurs in many areas of the world, whereas Yaws occurs in tropical areas of Africa, South America, the Caribbean, and Indonesia. Endemic syphilis is limited to arid areas of Africa and the Middle East.[145]

LABORATORY SAFETY

The agent may be present in materials collected from cutaneous and mucosal lesions and in blood. Accidental parenteral inoculation, contact with mucous membranes or broken skin with infectious clinical materials are the primary hazards to laboratory personnel.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or other clinical samples from humans or infected rabbits. Gloves should be worn when there is a likelihood of direct skin contact with infective materials. Periodic serological monitoring should be considered in personnel regularly working with these materials. ABSL-2 practices, containment equipment, and facilities are recommended for work with infected animals.

SPECIAL ISSUES

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Vibrio enteritis species (V. cholerae, V. parahaemolyticus)

Vibrio species are straight or curved motile gram-negative rods. Growth of *Vibrio* species is stimulated by sodium and the natural habitats of these organisms are primarily aquatic environments. Although 12 different *Vibrio* species have been isolated from clinical specimens, *V. cholerae* and *V. parahaemolyticus* are the best-documented causes of human disease.[148] Vibrios may cause either diarrhea or extraintestinal infections.

Occupational Infections

Rare cases of bacterial enteritis due to LAI with either *V. cholerae* or *V. parahaemolyticus* have been reported from around the world.[4] Naturally and experimentally infected animals[149] and shellfish[150,151] are potential sources for such illnesses.

Natural Modes of Infection

The most common natural mode of infection is the ingestion of contaminated food or water. The human oral infecting dose of *V. cholerae* in healthy non-achlorhydric individuals is approximately 10^6-10^{11} colony forming units,[152] while that of *V. parahaemolyticus* ranges from 10^5-10^7 cells.[153] The importance of aerosol exposure is unknown although it has been implicated in at least one instance.[149] The risk of infection following oral exposure is increased in persons with abnormal gastrointestinal physiology including individuals on antacids, with achlorhydria, or with partial or complete gastrectomies.[154]

LABORATORY SAFETY

Pathogenic vibrios can be present in human fecal samples, or in the meats and the exterior surfaces of marine invertebrates such as shellfish. Other clinical specimens from which vibrios may be isolated include blood, arm or leg wounds, eye, ear, and gall bladder.[148] Accidental oral ingestion of *V. cholerae* or *V. parahaemolyticus* principally results from hands contaminated from the use of syringes or the handling of naturally contaminated marine samples without gloves.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

SPECIAL ISSUES

Vaccines The reader is advised to consult the current recommendations of the ACIP published in the MMWR for vaccination recommendations against *V. cholera*. There are currently no human vaccines against *V. parahaemolyticus*.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Yersinia pestis

Yersinia pestis, the causative agent of plague, is a gram-negative, microaerophilic coccobacillus frequently characterized by a "safety pin" appearance on stained preparations from specimens. It is nonmotile and nonsporulating. There are three biotypes of *Y. pestis*, differentiated by their ability to ferment glycerol and reduce nitrate. All three biotypes are virulent. The incubation period for bubonic plague ranges from two to six days while the incubation period for pneumonic plague is one to six days. Pneumonic plague is transmissible person-to-person;[155] whereas bubonic plague is not. Laboratory animal studies have shown the lethal and infectious doses of *Y. pestis* to be quite low (less than 100 colony forming units).[156]

Occupational Infections

Y. pestis is a documented laboratory hazard. Prior to 1950, at least 10 laboratory-acquired cases were reported in the United States, four of which were fatal.[4,157] Veterinary staff and pet owners have become infected when handling domestic cats with oropharyngeal or pneumonic plague.

Natural Modes of Infection

Infective fleabites are the most common mode of transmission, but direct human contact with infected tissues or body fluids of animals and humans also may serve as sources of infection.

Primary pneumonic plague arises from the inhalation of infectious respiratory droplets or other airborne materials from infected animals or humans. This form of plague has a high case fatality rate if not treated and poses the risk of person-to-person transmission.

LABORATORY SAFETY

The agent has been isolated, in order of frequency of recovery, from bubo aspirate, blood, liver, spleen, sputum, lung, bone marrow, CSF, and infrequently from feces and urine, depending on the clinical form and stage of the disease. Primary hazards to laboratory personnel include direct contact with cultures and infectious materials from humans or animal hosts and inhalation of infectious aerosols or droplets generated during

their manipulation. Laboratory and field personnel should be counseled on methods to avoid fleabites and accidental autoinoculation when handling potentially infected live or dead animals.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the handling of potentially infectious clinical materials and cultures. In addition, because the infectious dose is so small, all work, including necropsies of potentially infected animals should be performed in a BSC. Special care should be taken to avoid generating aerosols or airborne droplets while handling infectious materials or when performing necropsies on naturally or experimentally infected animals. Gloves should be worn when handling potentially infectious materials including field or laboratory infected animals. BSL-3 is recommended for activities with high potential for droplet or aerosol production, and for activities involving large scale production or high concentrations of infectious materials. Resistance of *Y. pestis* strains to antibiotics used in the treatment of plague should be considered in a thorough risk assessment and may require additional containment for personal protective equipment. For animal studies, a risk assessment that takes into account the animal species, infective strain, and proposed procedures should be performed in order to determine if ABSL-2 or ABSL-3 practices, containment equipment, and facilities should be employed. BSL-3 facilities and arthropod containment level 3 practices are recommended for all laboratory work involving infected arthropods.[158] See Appendix G for additional information on arthropod containment guidelines.

SPECIAL ISSUES

Select Agent *Yersinis pestis* is an HHS Select Agent requiring registration with CDC for the possession, use, storage and transfer. See Appendix F for further information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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- **Section VIII-B: Fungal Agents**

Agent: Blastomyces dermatitidis

Blastomyces dermatitidis is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia) that are the infectious particles; these convert to large budding yeasts under the appropriate culture conditions *in vitro* at 37°C and in the parasitic phase *in vivo* in warm-blooded animals. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Three groups are at greatest risk of laboratory-acquired infection: microbiologists, veterinarians and pathologists.[1] Laboratory-associated local infections have been reported following accidental parenteral inoculation with infected tissues or cultures containing yeast forms of *B. dermatitidis*.[2-8] Pulmonary infections have occurred following the presumed inhalation of conidia from mold-form cultures; two persons developed pneumonia and one had an osteolytic lesion from which *B. dermatitidis* was cultured.[9,10] Presumably, pulmonary infections are associated only with sporulating mold forms.

Natural Modes of Infection

The fungus has been reported from multiple geographically separated countries, but is best known as a fungus endemic to North America and in association with plant material in the environment. Infections are not communicable, but require common exposure from a point source. Although presumed to dwell within the soil of endemic areas, *B. dermatitidis* is extremely difficult to isolate from soil. Outbreaks associated with the exposure of people to decaying wood have been reported.[11]

LABORATORY SAFETY

Yeast forms may be present in the tissues of infected animals and in clinical specimens. Parenteral (subcutaneous) inoculation of these materials may cause local skin infection and granulomas. Mold form cultures of *B. dermatitidis* containing infectious conidia, and processing of soil or other environmental samples, may pose a hazard of aerosol exposure.

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials, animal tissues, yeast-form cultures, and infected animals. BSL-3 practices, containment equipment, and facilities are required for handling sporulating mold-form cultures already identified as *B. dermatitidis* and soil or other environmental samples known or likely to contain infectious conidia.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Coccidioides immitis and Coccidioides posadasii

Coccidioides spp. is endemic to lower sonoran deserts of the western hemisphere including northern Mexico, southern Arizona, central and southern California, and west Texas. The original species (*C. immitis*) has been divided into *C. immitis* and *C. posadasii*.[12] These species are dimorphic fungal pathogens existing in nature and in laboratory cultures at room temperature as filamentous molds with asexual spores (single-cell arthroconidia three to five microns in size) that are the infectious particles that convert to spherules under the appropriate culture conditions *in vitro* at 37°C and *in vivo* in warm-blooded animals.

Occupational Infections

Laboratory-associated coccidioidomycosis is a documented hazard of working with sporulating cultures of *Coccidioides* spp.[13-15] Occupational exposure has also been associated in endemic regions with archeology[16] and high dust exposure.[17] Attack rates for laboratory and occupational exposure are higher than for ambient exposure when large numbers of spores are inhaled. Smith reported that 28 of 31 (90%) laboratory-associated infections in his institution resulted in clinical disease, whereas more than half of infections acquired in nature were asymptomatic.[18] Risk of respiratory infection from exposure to infected tissue or aerosols of infected secretions is very low. Accidental percutaneous inoculation has typically resulted in local granuloma formation.[19]

Natural Modes of Infection

Single spores can produce ambient infections by the respiratory route. Peak exposures occur during arid seasons. *Coccidioides* spp. grow in infected tissue as larger multicellular spherules, up to 70 microns in diameter and pose little or no risk of infection from direct exposure.

The majority of ambient infections is subclinical and results in life-long protection from subsequent exposures. The incubation period is one to three weeks and manifests as a community-acquired pneumonia with immunologically mediated fatigue, skin rashes, and joint pain. One of the synonyms for coccidioidomycosis is desert rheumatism. A small proportion of infections is complicated by hematogenous dissemination from the lungs to other organs, most frequently skin, the skeleton, and the meninges. Disseminated infection is much more likely in persons with cellular immunodeficiencies (AIDS, organ transplant recipient, lymphoma).

LABORATORY SAFETY

Because of their size, the arthroconidia are conducive to ready dispersal in air and retention in the deep pulmonary spaces. The much larger size of the spherule considerably reduces the effectiveness of this form of the fungus as an airborne pathogen.

Spherules of the fungus may be present in clinical specimens and animal tissues, and infectious arthroconidia in mold cultures and soil or other samples from natural sites. Inhalation of arthroconidia from environmental samples or cultures of the mold form is a serious laboratory hazard. Personnel should be aware that infected animal or human clinical specimens or tissues stored or shipped in such a manner as to promote germination of arthroconidia pose a theoretical laboratory hazard.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues. ABSL-2 practices, containment equipment, and facilities are recommended for experimental animal studies when the route of challenge is parenteral.

BSL-3 practices, containment equipment, and facilities are recommended for propagating and manipulating sporulating cultures already identified as *Coccidioides* spp. and for processing soil or other environmental materials known to contain infectious arthroconidia. Experimental animal studies should be done at BSL-3 when challenge is via the intranasal or pulmonary route.

SPECIAL ISSUES

Select Agent Some *Coccidioides* spp.are Select Agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Cryptococcus neoformans

Cryptococcus neoformans is a monomorphic fungal pathogen existing in nature, in laboratory cultures at room temperature and *in vivo* as a budding yeast. The sexual stage is grouped with the Basidiomycetes and is characterized by sparse hyphal formation with basidiospores. Both basidiospores and asexual yeasts are infectious.

Occupational Infections

Accidental inoculation of a heavy inoculum of *C. neoformans* into the hands of laboratory workers has occurred during injection or necropsy of laboratory animals.[20,21] Either a local granuloma or no lesion was reported, suggesting low pathogenicity by this route. Respiratory infections as a consequence of laboratory exposure have not been recorded.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with pigeon feces. Infections are not transmissible from person-to-person, but require common exposure via the respiratory route to a point source.

LABORATORY SAFETY

Accidental parenteral inoculation of cultures or other infectious materials represents a potential hazard to laboratory personnel, particularly to those who may be immunocompromised. Bites by experimentally infected mice and manipulations of infectious environmental materials (e.g., pigeon feces) may also represent a potential hazard to laboratory personnel. *C. neoformans* has been isolated from bedding of cages housing mice with pulmonary infection indicating the potential for contamination of cages and animal facilities by infected animals.[22] Reports of cutaneous cryptococcal infection following minor skin injuries suggests that localized infection may complicate skin injuries incurred in laboratories that handle *C. neoformans*.[23]

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended, for activities with known or potentially infectious clinical, environmental, or culture materials and with experimentally infected animals. This agent and any samples that may contain this agent should also be handled in a Class II BSC.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts under the appropriate culture conditions *in vitro* at 37°C and in the parasitic phase *in vivo*. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.[24-27] Pulmonary infections have resulted from handling mold form cultures.[28,29] Local infection has resulted from skin puncture during autopsy of an infected human,[30] from accidental needle inoculation of a viable culture,[31] and from spray from a needle into the eye.[32] Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers.[33] Conidia are resistant to drying and may remain viable for long periods of time. The small size of the infective conidia (less than 5 microns) is conducive to airborne dispersal and intrapulmonary retention. Work with experimental animals suggests that hyphal fragments are capable of serving as viable inocula.[24]

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with starling and bat feces. It has been isolated from soil, often in river valleys, between latitudes 45°N and 45°S. Histoplasmosis is naturally acquired by the inhalation of infectious particles, usually microconidia.[24] Infections are not transmissible from person-to-person, but require common exposure to a point source.

LABORATORY SAFETY

The infective stage of this dimorphic fungus (conidia) is present in sporulating mold form cultures and in soil from endemic areas. The yeast form in tissues or fluids from infected animals may produce local infection following parenteral inoculation or splash onto mucous membranes.

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, animal tissues and mold cultures, identifying cultures in routine diagnostic laboratories, and for inoculating experimental animals, regardless of route. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

BSL-3 practices, containment equipment, and facilities are recommended for propagating sporulating cultures of *H. capsulatum* in the mold form, as well as processing soil or other environmental materials known or likely to contain infectious conidia.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Sporothrix schenckii

Sporothrix schenckii is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts in the parasitic phase *in vivo*. The sexual stage is unknown.

Occupational Infections

Most cases of sporotrichosis are reported sporadically following accidental inoculation with contaminated material. Large outbreaks have been documented in persons occupationally or recreationally exposed to soil or plant material containing the fungus. However, *S. schenckii* has caused a substantial number of local skin or eye infections in laboratory personnel.[34] Most occupational cases have been associated with accidents and have involved splashing culture material into the eye,[35,36] scratching,[37] or injecting[38] infected material into the skin or being bitten by an experimentally infected animal.[39,40] Skin infections in the absence of trauma have resulted also from handling cultures[41-43] or necropsy of animals[44] without any apparent trauma.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with sphagnum moss and gardening, often involving sphagnum moss and traumatic implantation. Infections are not transmissible from person-to-person, but require common exposure to a point source. Rare respiratory and zoonotic infections occur. It is thought that naturally occurring lung disease results from inhalation.

LABORATORY SAFETY

Although localized skin and eye infections have occurred in an occupational setting, no pulmonary infections have been reported as a result from laboratory exposure. It should be noted that serious disseminated infections have been reported in immunocompromised persons.[45]

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of suspected clinical specimens, soil and vegetation, and experimental animal activities with *S. schenckii*. Gloves should be worn during manipulation of *S. schenckii* and when handling experimentally infected animals. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Dermatophytes (Epidermophyton, Microsporum, and Trichophyton)

The dermatophytes are biologically related species of the genera, *Epidermophyton, Microsporum,* and *Trichophyton* that exist as monomorphic pathogens in nature, in laboratory cultures at room temperature and *in vivo* as filamentous molds. The sexual stages, when known, are Ascomycetes with infectious ascospores. These fungi are distributed worldwide, with particular species being endemic in particular regions. The species are grouped by natural environment habitat as being primarily associated with humans (anthrophilic), other animals (zoophilic), or soil (geophilic).

Occupational Infections

Although skin, hair, and nail infections by these molds are among the most prevalent of human infections, the processing of clinical material has not been associated with laboratory infections. Infections have been acquired through contacts with naturally or experimentally infected laboratory animals (mice, rabbits, guinea pigs, etc.) and, occasionally, with handling cultures.[26,29,45,46]

Systemic dermatophytosis is a rare condition. Superficial chronic infections occur frequently among immunocompromised individuals as well as elderly and diabetic persons. Susceptible individuals should use extra caution.[47-50]

Natural Modes of Infection

Infections can be transmissible from person-to-person, or acquired from common exposure to a point source. The dermatophytes cause infection (dermatophytosis) by invading the keratinized tissues of living animals and are among the commonest infectious agents of humans. This fungal group encompasses members of three genera: *Epidermophyton, Microsporum,* and *Trichophyton*. The severity of infection depends on the infective species or strain, the anatomic site and other host factors. One of the most severe dermatophytoses is favus, a disfiguring disease of the scalp caused by *Trychophyton schoenleinii*.

LABORATORY SAFETY

Dermatophytes pose a moderate potential hazard to individuals with normal immune status. In the clinical laboratory setting, the inappropriate handling of cultures is the most common source of infection for laboratory personnel. The most common laboratory procedure for detection of the infective dermatophyte is the direct microscopic

examination of contaminated skin, hair, and nails, followed by its isolation and identification on appropriated culture media. Direct contact with contaminated skin, hair, and nails of humans could be another source of infection.[48,49] In research laboratories, dermatophytosis can be acquired by contact with contaminated soil (source of infection: geophilic species) or animal hosts (source of infection: zoophilic species).

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling cultures and soil samples. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Miscellaneous Molds

Several molds have caused serious infection in immunocompetent hosts following presumed inhalation or accidental subcutaneous inoculation from environmental sources. These agents include the dimorphic mold, *Penicillium marneffei*, and the dematiaceous (brown-pigmented) molds, *Bipolaris* species, *Cladophialophora bantiana, Exophiala (Wangiella) dermatitidis, Exserohilum* species, *Fonsecaea pedrosoi, Ochroconis gallopava (Dactylaria gallopava), Ramichloridium mackenziei (Ramichloridium obovoideum), Rhinocladiella atrovirens,* and *Scedosporium prolificans*.[51]

Occupational Infections

Even though no laboratory-acquired infections appear to have been reported with most of these agents, the gravity of naturally-acquired illness is sufficient to merit special precautions in the laboratory. *Penicillium marneffei* has caused a localized infection in a laboratory worker.[52] It also caused a case of laboratory-acquired disseminated infection following presumed inhalation when an undiagnosed HIV-positive individual visited a laboratory where students were handling cultures on the open bench.[53]

Natural Modes of Infection

The natural mode of infection varies by specific species; most are poorly characterized.

LABORATORY SAFETY

Inhalation of conidia from sporulating mold cultures or accidental injection into the skin during infection or experimental animals is a potential risk to laboratory personnel.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for propagating and manipulating cultures known to contain these agents. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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- **Section VIII-C: Parasitic Agents**

General Issues

Additional details about occupationally-acquired cases of parasitic infections, as well as recommendations for postexposure management, are provided elsewhere.[1-3] Effective antimicrobial treatment is available for most parasitic infections.[4] Immunocompromised persons should receive individualized counseling (specific to host and parasite factors) from their personal healthcare provider and their employer about the potential risks associated with working with live organisms.

BSL-2 and ABSL-2 practices,[5] containment equipment, and facilities are recommended for activities with infective stages of the parasites discussed in this chapter.

Microsporidia, historically considered parasites, are now recognized by most experts to be fungi; however, Microsporidia are maintained in the parasitic agent section is this edition. These organisms are discussed here because a laboratory-acquired case of infection has been reported, and most persons currently still look for Microsporidia associated with discussion of parasitic agents.

Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Agent: Blood and Tissue Protozoal Parasites

Blood and tissue protozoal parasites include *Babesia microti*, *Leishmania (Leishmania) donovani*, *L. (Viannia) braziliensi*, *L. (Leishmania) tropica*, *L. (Leishmania) mexicana*, *L. (Leishmania) amazonensis*, *L. (Viannia) guyanensis*, *Plasmodium falciparum*, *Plasmodium cynomolgi*, *P. vivax*, *Toxoplasma gondii*, *Trypanosoma cruzi*, *Trypanosoma brucei gambiense*, *T. b. rhodesiense*.

Leishmania spp. cause human leishmaniasis; *Plasmodium falciparum* and *P. vivax* cause human malaria, and *P. cynomolgi* is one of several causes of NHP malaria; *Toxoplasma gondii* causes toxoplasmosis; *Trypanosoma cruzi* causes American trypanosomiasis or Chagas disease; and *Trypanosoma brucei gambiense* and *T. b. rhodesiense* cause African trypanosomiasis or (African) sleeping sickness. With the exception of *Leishmania* and *Toxoplasma*, these agents are classically thought of as bloodborne and have stages that circulate in the blood. Although not always recognized, both *Leishmania* and *Toxoplasma* may have stages that circulate in the blood. Some, such as *Plasmodium* and *Trypanosoma cruzi*, also have tissue stages. *Leishmania* spp. are well recognized to have skin and deep tissue stages and *Toxoplasma gondii* forms tissue cysts, including in the central nervous system.

Occupational Infections

Laboratory-acquired infections with *Leishmania* spp., *Plasmodium* spp., *Toxoplasma gondii*, and *Trypanosoma* spp. have been reported; the majority of these involved needle-stick or other cutaneous exposure to infectious stages of the organisms through abraded skin, including microabrasions.[1]

Laboratory-acquired infections may be asymptomatic. If clinically manifest, they may exhibit features similar to those seen in naturally acquired infections, although bypassing natural modes of infection could result in atypical signs and symptoms. Cutaneous leishmaniasis could manifest as various types of skin lesions (e.g., nodules, ulcers, plaques), while visceral leishmaniasis may result in fever, hepatosplenomegaly, and pancytopenia. However, only one of the four laboratorians known to have become infected with *L. (L.) donovani*, an organism typically associated with visceral leishmaniasis, developed clinical manifestations of visceral involvement (e.g., fever, splenomegaly, leukopenia).[1] The other three laboratorians developed skin lesions. Laboratory-acquired malaria infections may result in fever and chills, fatigue, and hemolytic anemia. Laboratorians can become infected with *T. gondii* through accidental ingestion of sporulated oocysts, but also may become infected through skin or mucous membrane contact with either tachyzoites or bradyzoites in human or animal tissue or culture. Symptoms in laboratory-acquired *T. gondii* infections may be restricted to flu-like conditions with enlarged lymph nodes, although rash may be present. *Trypanosoma cruzi* infection could manifest initially as swelling and redness at the inoculation site, fever, rash, and adenopathy. Myocarditis and electrocardiographic changes may develop. Infection with *T. b. rhodesiense* and *T. b. gambiense* also may cause initial swelling and redness at the inoculation site, followed by fever, rash, adenopathy, headache, fatigue and neurologic signs.

Blood and tissue protozoal infections associated with exposure to laboratory animals are not common. Potential direct sources of infection for laboratory personnel include accidental needle-stick while inoculating or bleeding animals, contact with lesion material from cutaneous leishmaniasis, and contact with blood of experimentally or naturally infected animals. In the case of rodents experimentally inoculated with *Toxoplasma gondii* via the intraperitoneal route, contact with peritoneal fluid could result in exposure to infectious organisms. Mosquito-transmitted malaria infections can occur under laboratory conditions, and contact with body fluids (including feces) of reduviids (triatomines) experimentally or naturally infected with *T. cruzi* poses a risk to laboratory personnel.

Babesia microti and other *Babesia* spp. can cause human babesiosis or piroplasmosis. Under natural conditions, *Babesia* is transmitted by the bite of an infected tick; in the United States, hard ticks (*Ixodes)* are the principle vectors. Although no laboratory infections with *Babesia* have been reported, they could easily result from accidental needle-stick or other cutaneous exposure of abraded skin to blood containing parasites. Persons who are asplenic, immunocompromised, or elderly have increased risk for severe illness if infected.

Natural Modes of Infection

Leishmaniasis is endemic in parts of the tropics, subtropics, and southern Europe, while malaria is widely distributed throughout the tropics. However, the prevalence of these diseases varies widely among endemic areas and they can be very focal in nature. The four species of malaria that infect humans have no animal reservoir hosts. Some *Leishmania* spp. may have a number of important mammalian reservoir hosts, including rodents and dogs. Only cats and other felines can serve as definitive hosts for *Toxoplasma gondii*, which is distributed worldwide. Birds and mammals, including sheep, pigs, rodents, cattle, deer, and humans can be infected from ingestion of tissue cysts or fecal oocysts and subsequently develop tissue cysts throughout the body. Chagas disease occurs from Mexico southward throughout most of Central and South America, with the exception of the southern-most tip of South America. It has been characterized in some accounts as a zoonotic infection, yet the role of animals in maintaining human infection is unclear. A variety of domestic and wild animals is found naturally infected with *T. cruzi*, but human infection undoubtedly serves as the major source of infection for other humans. African trypanosomiasis is endemic in sub-Saharan Africa but is extremely focal in its distribution. Generally, *T. b. gambiense* occurs in West and Central Africa while *T. b. rhodesiense* occurs in East and Southeast Africa. *T. b. rhodesiense* is a zoonotic infection with cattle and game animals serving as reservoir hosts, whereas humans are the only epidemiologically important hosts for *T. b. gambiense*.

Leishmania, *Plasmodium*, and both American and African trypanosomes are all transmitted in nature by blood-sucking insects. Sandflies in the genera *Phlebotomus* and *Lutzomyia* transmit *Leishmania*; mosquitoes in the genus *Anopheles* transmit *Plasmodium*; reduviid (triatomine) bugs such as *Triatoma*, *Rhodnius*, and *Panstrongylus* transmit *T. cruzi* (in the feces rather than the saliva of the bug), and tsetse flies in the genus *Glossina* transmit African trypanosomes.

LABORATORY SAFETY

Infective stages may be present in blood, CSF, bone marrow, or other biopsy tissue, lesion exudates, and infected arthropods. Depending on the parasite, the primary laboratory hazards are skin penetration through wounds or microabrasions, accidental parenteral inoculation, and transmission by arthropod vectors. Aerosol or droplet exposure of organisms to the mucous membranes of the eyes, nose, or mouth are potential hazards when working with cultures of *Leishmania*, *Toxoplasma gondii*, or *T. cruzi*, or with tissue homogenates or blood containing hemoflagellates. Immunocompromised persons should avoid working with live organisms.

Because of the potential for grave consequences of toxoplasmosis in the developing fetus, women who might become pregnant should receive counseling from their personal physician. Working with infectious oocysts poses the greatest risk of acquiring infection; needle-sticks with material containing tachyzoites or bradyzoites also pose a significant risk. Infection with tachyzoites or bradyzoites through mucous membranes or skin abrasions is also possible. Kittens and cats that might be naturally

infected with *Toxoplasma* pose some risk to personnel.[3] Good hygiene and use of personal protection measures would reduce the risk.

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed. Infected arthropods should be maintained in facilities that reasonably preclude the exposure of personnel or the escape of insects (See Appendix E). Personal protection (e.g., lab coat, gloves, face shield), in conjunction with containment in a BSC, is indicated when working with cultures, tissue homogenates, or blood containing organisms.

SPECIAL ISSUES

Treatment Highly effective medical treatment for most protozoal infections exists.[2] An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Intestinal Protozoal Parasites

Cryptosporidium parvum, C. hominis, and *Isospora belli* cause intestinal coccidiosis, most often referred to as cryptosporidiosis and isosporiasis, respectively. *Entamoeba histolytica* can cause both intestinal and extraintestinal infection called amebiasis, and *Giardia intestinalis* causes giardiasis.

Occupational Infections

Laboratory-acquired infections with *Cryptosporidium* spp., *E. histolytica, G. intestinalis,* and *I. belli* have been reported.[1] The mode of exposure in laboratory-acquired infections in this group of agents mimics the natural infection routes for the most part, and consequently, clinical symptoms are typically very similar to those seen in naturally acquired infections. For *Cryptosporidium*, *E. histolytica*, *G. intestinalis*, and *I. belli*, the common clinical manifestations are symptoms of gastroenteritis, e.g., diarrhea, abdominal pain and cramping, loss of appetite. Infection with *E. histolytica* may result in bloody stools.

Laboratory animal-associated infections with this group of organisms have been reported and provide a direct source of infection for laboratory personnel who are exposed to feces of experimentally or naturally infected animals.[3] Handling *Cryptosporidium* oocysts requires special care, as laboratory-acquired infections have occurred commonly in personnel working with this agent, especially if calves are used as

the source of oocysts. Other experimentally infected animals pose potential risks as well. Circumstantial evidence suggests that airborne transmission of oocysts of this small organism (i.e., 4-6 μm diameter) may occur. Rigid adherence to protocol should reduce the occurrence of laboratory-acquired infection in laboratory and animal care personnel.

Natural Modes of Infection

All of these intestinal protozoa have a cosmopolitan distribution, and in some settings, including developed countries, the prevalence of infection can be high. The natural mode of infection for this group of organisms is typically ingestion of an environmentally hardy oocyst (for the coccidia) or cyst (for *E. histolytica* and *G. intestinalis*). The ID50, best established for *Cryptosporidium*, has been shown for some strains to be 5-10 oocysts. This suggests that even a single oocyst might pose a risk for infection in an exposed laboratorian. The infectious dose for other parasites in this group is not as well established, but is probably in the same range. Further, because these protozoa multiply in the host, ingestion of even small inocula can cause infection and illness. The role for animal reservoir hosts is diverse in this group of organisms. In the case of *C. hominis*, principally humans are infected, whereas for *C. parvum*, humans, cattle, and other mammals can be infected and serve as reservoir hosts for human infection. In the case of *E. histolytica*, humans serve as the only significant source of infection, and there is no convincing evidence that any animal serves as reservoir host for *I. belli*. The extent to which *Giardia* spp. parasitizing animals can infect humans is only now becoming better understood, but most human infection seem to be acquired from human-to-human transmission. The organisms in this group do not require more than one host to complete their life cycle because they infect, develop, and result in shedding of infectious stages all in a single host. Ingestion of contaminated or recreational water has also been a source of Giardiasis.

LABORATORY SAFETY

Infective stages may be present in the feces or other body fluids and tissues. Depending on the parasite, ingestion is the primary laboratory hazard. Immunocompromised persons should avoid working with live organisms. Laboratorians who work only with killed or inactivated parasite materials, or parasite fractions, are not at significant risk.

No laboratory infections with Microsporidia have been reported, but they could result from ingestion of spores in feces, urine, sputum, CSF, or culture. Similarly, no accidental laboratory infection with *Sarcocystis* has been reported, although care should be exercised when working with infected meat products to avoid accidental ingestion. It is not known if laboratorians could be accidentally infected through parenteral inoculation of *Sarcocystis*; nevertheless caution should be exercised when working with cultures, homogenates, etc. No laboratory-acquired infections have been reported with *Acanthamoeba* spp., *Balamuthia mandrillaris* or *Naegleria fowleri*; however, the possibility of becoming infected by inhalation, by accidental needle-sticks, or through exposure to mucous membranes or microabrasions of the skin should be considered.

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed. Primary containment (e.g., BSC) or personal protection (e.g., face shield) is especially important when working with *Cryptosporidium.* Oocysts are infectious when shed (i.e., are already sporulated and do not require further development time outside the host), often are present in stool in high numbers, and are environmentally hardy.

SPECIAL ISSUES

Treatment Highly effective medical treatment exists for most protozoal infections with the exception of *Cryptosporidium*.[2]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Trematode Parasites

Fasciola hepatica, the sheep liver fluke, causes fascioliasis, where the adult flukes live in the common and hepatic bile ducts of the human or animal host. *Schistosoma mansoni*, causes intestinal schistosomiasis or bilharziasis, also known as Manson's blood fluke, in which the adult flukes reside in the venules of the bowel and rectum.

Occupational Infections

Laboratory-acquired infections with *F. hepatica* and *S. mansoni* have been reported.[1] By nature of the infection, none have been directly associated with laboratory animals, with the exception of infected mollusk intermediate hosts.

Laboratory-acquired infections with *F. hepatica* are generally asymptomatic, but could have clinical manifestations such as right upper quadrant pain, biliary colic, obstructive jaundice, elevated transaminase levels, and other pathology associated with hepatic damage resulting from migration of the fluke through the liver en route to the bile duct. Most laboratory exposures to schistosomes would result in predictably low worm burdens with minimal disease potential. However, clinical manifestations of infection with *S. mansoni* could include dermatitis, fever, cough, hepatosplenomegaly, and adenopathy.

Natural Modes of Infection

Fasciola hepatica has a cosmopolitan distribution and is most common in sheep-raising areas, although other natural hosts include goats, cattle, hogs, deer, and rodents.

Snails in the family Lymnaeidae, primarily species of *Lymnaea*, are intermediate hosts for *F. hepatica*, and release cercariae that encyst on vegetation. Persons become infected with *F. heptica* by eating raw or poorly cooked vegetation, especially green leafy plants such as watercress, on which metacercariae have encysted.

Schistosoma mansoni is widely distributed in Africa, South America, and the Caribbean; the prevalence of infection has been rapidly changing in some areas. Infection occurs when persons are exposed to free-swimming cercariae in contaminated bodies of water; cercariae can penetrate intact skin. The natural snail hosts capable of supporting development of *S. mansoni* are various species of *Biomphalaria*, although other species of snails have been reported to be naturally infected, and still others have been experimentally infected.

LABORATORY SAFETY

Infective stages of *F. hepatica* (metacercariae) and *S. mansoni* (cercariae) may be found, respectively, encysted on aquatic plants or in the water in laboratory aquaria used to maintain snail intermediate hosts. Ingestion of fluke metacercariae and skin penetration by schistosome cercariae are the primary laboratory hazards. Dissection or crushing of schistosome-infected snails may also result in exposure of skin or mucous membrane to cercariae-containing droplets. Additionally, metacercariae may be inadvertently transferred from hand to mouth by fingers or gloves, following contact with contaminated aquatic vegetation or aquaria.

All reported cases of laboratory-acquired schistosomiasis have been caused by *S. mansoni*, which probably reflects the fact that many more laboratories work with *S. mansoni* than with other *Schistosoma* spp. However, accidental infection with *S. haematobium*, *S. japonicum*, and *S. mekongi* could easily occur in the same manner as described for *S. mansoni*.

Exposure to cercariae of non-human species of schistosomes (e.g., avian species) may cause mild to severe dermatitis (swimmer’s itch).

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment and facilities are recommended for laboratory work with infective stages of the parasites listed. Gloves should be worn when there may be direct contact with water containing cercariae or vegetation with encysted metacercariae from naturally or experimentally infected snail intermediate hosts. Long-sleeved laboratory coats or other protective garb should be worn when working in the immediate area of aquaria or other water sources that may contain schistosome cercariae. Water from laboratory aquaria containing snails and cercariae should be decontaminated before discharged to sanitary sewers.

SPECIAL ISSUES

Treatment Highly effective medical treatment for most trematode infections exists.[2]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Cestode Parasites

Cestode parasites include *Echinococcus spp.*, *Hymenolepis nana*, and *Taenia solium.* Echinococcosis is an infection caused by cestodes in the genus *Echinococcus*; *E. granulosus* causes cystic echinococcosis, *E. multilocularis* causes alveolar echinococcosis, and *E. vogeli* and *E. oligarthrus* cause polycystic echinococcosis. Humans serve as intermediate hosts and harbor the metacestode or larval stage, which produces a hydatid cyst. *Hymenolepis nana*, the dwarf tapeworm, is cosmopolitan in distribution and produces hymenolepiasis, or intestinal infection with the adult tapeworm. *Taenia solium*, the pork tapeworm, causes both taeniasis (infection of the intestinal tract with the adult worm), and cysticercosis (infection of subcutaneous, intermuscular, and central nervous system with the metacestode stage or cysticercus).

Occupational Infections

No laboratory-acquired infections have been reported with any cestode parasite.

Natural Modes of Infection

The infectious stage of *Echinococcus, Hymenolepis*, and *Taenia* is the oncosphere contained within the egg. *Hymenolepis nana* is a one-host parasite and does not require an intermediate host; it is directly transmissible by ingestion of feces of infected humans or rodents. The life cycles of *Echinococcus* and *Taenia* require two hosts. Canids, including dogs, wolves, foxes, coyotes, and jackals, are the definitive hosts for *E. granulosus*, and various herbivores such as sheep, cattle, deer, and horses are the intermediate hosts. Foxes and coyotes are the principle definitive hosts for *E. multilocularis*, although dogs and cats also can become infected and rodents serve as the intermediate hosts. Bush dogs and pacas serve as the definitive and intermediate hosts, respectively, for *E. vogeli*. Dogs also may be infected. *Echinococcus oligarthrus* uses wild felines, including cougar, jaguarondi, jaguar, ocelot, and pampas cat, as definitive hosts and various rodents such as agoutis, pacas, spiny rats, and rabbits serve as intermediate hosts. People become infected when eggs shed by the definitive host are accidentally ingested. For *T. solium*, people can serve both as definitive host (harbor the adult tapeworm), and as accidental intermediate host (harbor the larval stages cysticerci). Pigs are the usual intermediate host, becoming infected as they scavenge human feces containing eggs.

LABORATORY SAFETY

Infective eggs of *Echinococcus* spp. may be present in the feces of carnivore definitive hosts.[3] *Echinococcus granulosus* poses the greatest risk because it is the most common and widely distributed species, and because dogs are the primary definitive hosts. For *T. solium*, infective eggs in the feces of humans serve as the source of infection. Accidental ingestion of infective eggs from these sources is the primary laboratory hazard. Ingestion of cysticerci of *T. solium (Cysticercus cellulosae)* leads to human infection with the adult tapeworm. For those cestodes listed, the ingestion of a single infective egg from the feces of the definitive host could potentially result in serious disease. Ingestion of the eggs of *H. nana* in the feces of definitive hosts (humans or rodents) could result in intestinal infection.

Although no LAI with either *Echinococcus* spp. or *T. solium* have been reported, the consequences of such infections could be serious. Laboratory-acquired infections with cestodes could result in various clinical manifestations, depending upon the type of cestode. Human infection with *Echinococcus* spp. could range from asymptomatic to severe. The severity and nature of the signs and symptoms depends upon the location of the cysts, their size, and condition (alive versus dead). Clinical manifestations of a liver cyst could include hepatosplenomegaly, right epigastric pain, and nausea, while a lung cyst may cause chest pain, dyspnea, and hemoptysis. Infection with *H. nana* may cause abdominal pain and diarrhea. For *T. solium*, ingestion of eggs from human feces can result in cysticercosis, with cysts located in subcutaneous and intermuscular tissues, where they may be asymptomatic. Cysts in the central nervous system may cause seizures and other neurologic symptoms. Ingestion of tissue cysts of *T. solium* can lead to development of adult worms in the intestine of humans. Immunocompromised persons working with these cestodes must take special care as the asexual multiplication of the larval stages of these parasites makes them especially dangerous to such persons.

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for work with infective stages of these parasites. Special attention should be given to personal hygiene (e.g., hand washing) and laboratory practices that would reduce the risk of accidental ingestion of infective eggs. Gloves are recommended when there may be direct contact with feces or with surfaces contaminated with fresh feces of carnivores infected with *Echinococcus* spp., humans infected with *T. solium*, or humans or rodents infected with *H. nana*.

SPECIAL ISSUES

Treatment Highly effective medical treatment for most cestode infections exists.[2]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Nematode Parasites

Ancylostoma braziliense and *A. caninum* cause hookworm infection in cats and dogs, respectively. *Ascaris lumbricoides* causes ascariasis and is known as the large intestinal roundworm of humans. *Enterobius vermicularis*, known as the human pinworm or seatworm, causes enterobiasis or oxyuriasis. *Strongyloides*, the threadworm, causes strongyloidiasis. *Ancylostoma* spp., *A. lumbricoides*, and *Strongyloides* spp. reside as adults in the small intestine of their natural hosts, whereas *E. vermicularis* colonizes the cecum and appendix.

Occupational Infections

Laboratory associated infections with *Ancylostoma* spp., *A. lumbricoides*, *E. vermicularis*, and *Strongyloides* spp. have been reported.[1] In addition, laboratory infections with hookworms and *Strongyloides* presumptively acquired from infected animals have been reported. The infective larvae in the feces of dogs, cats, primates, and other laboratory animals create a potential hazard for laboratory staff and animal care personnel.[3] Allergic reactions to various antigenic components of human and animal ascarids (e.g., aerosolized antigens) may pose risk to sensitized persons.

Laboratory-acquired infections with these nematodes can be asymptomatic, or can present with a range of clinical manifestations dependent upon the species and their location in host. Infection from hookworm with animal species can result in cutaneous larva migrans or creeping eruption of the skin. Infection with *A. lumbricoides* may produce cough, fever, and pneumonitis as larvae migrate through the lung, followed by abdominal cramps and diarrhea or constipation from adult worms in the intestine. Infection with *E. vermicularis* usually causes perianal pruritis, with intense itching. Infection with animal *Strongyloides* spp. may induce cutaneous larva migrans.

Natural Modes of Infection

Ancylostoma infection in dogs and cats is endemic worldwide. Human infection occurs through penetration of the skin. Cutaneous larva migrans or creeping eruption occurs when infective larvae of animal hookworms, typically dog and cat hookworms, penetrate the skin and begin wandering. *Ancylostoma* larvae can also cause infection if ingested. These larvae do not typically reach the intestinal tract, although *A. caninum* has on rare occasions developed into non-gravid adult worms in the human gut.

Ascaris lumbricoides infection is endemic in tropical and subtropical regions of the world. Infection occurs following accidental ingestion of infective eggs. Unembryonated eggs passed in the stool require two to three weeks to become infectious, and *Ascaris* eggs are very hardy in the environment.

Enterobius vermicularis occurs worldwide, although infection tends to be more common in school-age children than adults, and in temperate than in tropical regions. Pinworm infection is acquired by ingestion of infective eggs, most often on contaminated fingers following scratching of the perianal skin. Eggs passed by female worms are not immediately infective, but only require several hours' incubation to become fully infectious. Infection with this worm is relatively short (60 days on average), and reinfection is required to maintain an infection.

Strongyloides infection in animals is endemic worldwide. People become infected with animal *Strongyloides* when infective, filariform larvae penetrate the skin, and can develop cutaneous creeping eruption (larva currens).

LABORATORY SAFETY

Eggs and larvae of most nematodes are not infective in freshly passed feces; development to the infective stages may require from one day to several weeks. Ingestion of the infective eggs or skin penetration by infective larvae are the primary hazards to laboratory staff and animal care personnel. Development of hypersensitivity is common in laboratory personnel with frequent exposure to aerosolized antigens of ascarids.

Ascarid eggs are sticky, and special care should be taken to ensure thorough cleaning of contaminated surfaces and equipment. Caution should be used even when working with formalin-fixed stool samples because ascarid eggs can remain viable and continue to develop to the infective stage in formalin.[4]

Working with infective eggs of other ascarids, such as *Toxocara* and *Baylisascaris*, poses significant risk because of the potential for visceral migration of larvae, including invasion of the eyes and central nervous system. *Strongyloides stercoralis* is of particular concern to immunosuppressed persons because potentially life-threatening systemic hyperinfection can occur. Lugol's iodine kills infective larvae and should be sprayed onto skin or laboratory surfaces that were contaminated accidentally. The larvae of *Trichinella* in fresh or digested tissue could cause infection if accidentally ingested. Arthropods infected with filarial parasites pose a potential hazard to laboratory personnel.

Containment Recommendations

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the nematodes listed here. Exposure to aerosolized sensitizing antigens of ascarids should be avoided. Primary containment (e.g., BSC) is recommended for work that may result in aerosolization of sensitization from occurring.

SPECIAL ISSUES

Treatment Highly effective medical treatment for most nematode infections exists.[2]

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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- **Section VIII-D: Rickettsial Agents**

Agent: Coxiella burnetii

Coxiella burnetii is the etiologic agent of Q fever. *C. burnetii* is a bacterial obligate intracellular pathogen that undergoes its developmental cycle within an acidic vacuolar compartment exhibiting many characteristics of a phagolysosome. The developmental cycle consists of a large (approximately 1 µm in length) cell variant that is believed to be the more metabolically active, replicative cell type and a smaller, more structurally stable cell variant that is highly infectious and quite resistant to drying and environmental conditions.[1-4] The organism undergoes a virulent (Phase I) to avirulent (Phase II) transition upon serial laboratory passage in eggs or tissue culture.

The infectious dose of virulent Phase I organisms in laboratory animals has been calculated to be as small as a single organism.[5] The estimated human infectious dose for Q fever by inhalation is approximately 10 organisms.[6] Typically, the disease manifests with flu-like symptoms including fever, headache, and myalgia but can also cause pneumonia and hepatomegaly. Infections range from sub-clinical to severe although primary infections respond readily to antibiotic treatment. Although rare, *C. burnetii* is known to cause chronic infections such as endocarditis or granulomatous hepatitis.[7]

Occupational Infections

Q fever is the second most commonly reported LAI in Pike's compilation. Outbreaks involving 15 or more persons were recorded in several institutions.[8,9] Infectious aerosols are the most likely route of laboratory-acquired infections. Experimentally infected animals also may serve as potential sources of infection for laboratory and animal care personnel. Exposure to naturally infected, often asymptomatic, sheep and their birth products is a documented hazard to personnel.[10,11]

Natural Modes of Infection

Q fever (Q for query) occurs worldwide. Broad ranges of domestic and wild mammals are natural hosts for Q fever and sources of human infection. Parturient animals and their birth products are common sources of infection. The placenta of infected sheep may contain as many as 10^9 organisms per gram of tissue[12] and milk may contain 10^5 organisms per gram. The resistance of the organism to drying and its low infectious dose can lead to dispersal from contaminated sites.

LABORATORY SAFETY

The necessity of using embryonated eggs or cell culture techniques for the propagation of *C. burnetii* leads to extensive purification procedures. Exposure to infectious aerosols and parenteral inoculation cause most infections in laboratory and animal care personnel.[8,9] The agent may be present in infected arthropods and in the blood, urine, feces, milk, and tissues of infected animals or human hosts. Exposure to

naturally infected, often asymptomatic, sheep and their birth products is a documented hazard to personnel.[10,11] Recommended precautions for facilities using sheep as experimental animals are described elsewhere.[10,13]

Containment Recommendations

BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears. BSL-3 practices and facilities are recommended for activities involving the inoculation, incubation, and harvesting of embryonated eggs or cell cultures, the necropsy of infected animals and the manipulation of infected tissues. Experimentally infected animals should be maintained under ABSL-3 because infected rodents may shed the organisms in urine or feces.[8] A specific plaque-purified clonal isolate of an avirulent (Phase II) strain (Nine Mile) may be safely handled under BSL-2 conditions.[14]

SPECIAL ISSUES

Vaccines An investigational Phase I, Q fever vaccine (IND) is available on a limited basis from the Special Immunizations Program (301-619-4653) of the USAMRIID, Fort Detrick, Maryland, for at-risk personnel under a cooperative agreement with the individual's requesting institution. The use of this vaccine should be restricted to those who are at high risk of exposure and who have no demonstrated sensitivity to Q fever antigen. The vaccine can be reactogenic in those with prior immunity, thus requires skin testing before administration. The vaccine is only administered at USAMRIID and requires enrollment in their Q fever IND Immunization Program. For at-risk laboratory workers to participate in this program, fees are applicable. Individuals with valvular heart disease should not work with *C. burnetii* (See Chapter 7).

Select Agent *C. burnetii* is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agents: Rickettsia prowazekii, Rickettsia typhi (R. mooseri), Orientia (Rickettsia) tsutsugamushi and Spotted Fever Group agents of human disease; Rickettsia rickettsii, Rickettsia conorii, Rickettsia akari, Rickettsia australis, Rickettsia siberica, and Rickettsia japonicum

Rickettsia prowazekii, Rickettsia typhi (R. mooseri), Orientia (Rickettsia) tsutsugamushi and the Spotted Fever Group agents of human disease (*Rickettsia rickettsii, Rickettsia conorii, Rickettsia akari, Rickettsia australis, Rickettsia siberica*, and *Rickettsia japonicum)* are the etiologic agents of epidemic typhus, endemic (murine)

typhus), scrub typhus, Rocky Mountain spotted fever, Mediterranean spotted fever, rickettsialpox, Queensland tick typhus, and North Asian spotted fever, respectively.

Rickettsia spp. are bacterial obligate intracellular pathogens that are transmitted by arthropod vectors and replicate within the cytoplasm of eukaryotic host cells. Two groups are recognized within the genus, the typhus group and the spotted fever group. The more distantly related scrub typhus group is now considered a distinct genus, *Orientia.* Rickettsiae are primarily associated with arthropod vectors in which they may exist as endosymbionts that infect mammals, including humans, through the bite of infected ticks, lice, or fleas.[15]

Occupational Infections

Pike reported 57 cases of laboratory-associated typhus (type not specified), 56 cases of epidemic typhus with three deaths, and 68 cases of murine typhus.[8] Three cases of murine typhus have been reported from a research facility.[16] Two were associated with handling of infectious materials on the open bench; the third case resulted from an accidental parenteral inoculation. These three cases represented an attack rate of 20% in personnel working with infectious materials. Rocky Mountain spotted fever is a documented hazard to laboratory personnel. Pike reported 63 laboratory-associated cases, 11 of which were fatal.8 Oster reported nine cases occurring over a six year period in one laboratory. All were believed to have been acquired as a result of exposure to infectious aerosols.[17]

Natural Modes of Infection

The epidemiology of rickettsial infections reflects the prevalence of rickettsiae in the vector population and the interactions of arthropod vectors with humans. Epidemic typhus is unusual among rickettsiae in that humans are considered the primary host. Transmission is by the human body louse; thus, outbreaks are now associated with breakdowns of social conditions. Endemic typhus is maintained in rodents and transmitted to humans by fleas. The various spotted fever group rickettsiae are limited geographically, probably by the distribution of the arthropod vector, although specific spotted fever group rickettsiae are found on all continents.[15]

LABORATORY SAFETY

The necessity of using embryonated eggs or cell culture techniques for the propagation of *Rickettsia* spp. incorporates extensive purification procedures. Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of LAI.[18] Aerosol transmission of *R. rickettsii* has been experimentally documented in nonhuman primates.[19] Five cases of rickettsialpox recorded by Pike were associated with exposure to bites of infected mites.[8] Naturally and experimentally infected mammals, their ectoparasites, and their infected tissues are potential sources of human infection. The organisms are relatively unstable under ambient environmental conditions.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for nonpropagative laboratory procedures, including serological and fluorescent antibody procedures, and for the staining of impression smears. BSL-3 practices, containment equipment, and facilities are recommended for all other manipulations of known or potentially infectious materials, including necropsy of experimentally infected animals and trituration of their tissues, and inoculation, incubation, and harvesting of embryonated eggs or cell cultures. ABSL-2 practices, containment equipment, and facilities are recommended for the holding of experimentally infected mammals other than arthropods. BSL-3 practices, containment equipment, and facilities are recommended for animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease (See Appendix E).

Several species, including *R. montana*, *R. rhipicephali*, *R. belli*, and *R. canada*, are not known to cause human disease and may be handled under BSL-2 conditions. New species are being described frequently and should be evaluated for appropriate containment on a case-by-case basis. Because of the proven value of antibiotic therapy in the early stages of ricketsial infection, it is essential that laboratories have an effective system for reporting febrile illnesses in laboratory personnel, medical evaluation of potential cases and, when indicated, institution of appropriate antibiotic therapy.

Medical Response:

Under natural circumstances, the severity of disease caused by rickettsial agents varies considerably. In the laboratory, very large inocula are possible, which might produce unusual and perhaps very serious responses. Surveillance of personnel for laboratory-associated infections with rickettsial agents can dramatically reduce the risk of serious consequences of disease. Experience indicates that infections adequately treated with specific anti-rickettsial chemotherapy on the first day of disease do not generally present serious problems. However, delay in instituting appropriate chemotherapy may result in debilitating or severe acute disease ranging from increased periods of convalescence in typhus and scrub typhus to death in *R. rickettsii* infections. The key to reducing the severity of disease from laboratory-associated infections is a reliable medical response which includes: 1) round-the-clock availability of an experienced medical officer, 2) indoctrination of all personnel on the potential hazards of working with rickettsial agents and advantages of early therapy, 3) a reporting system for all recognized overt exposures and accidents, 4) the reporting of all febrile illnesses, especially those associated with headache, malaise, and prostration when no other certain cause exists, and 5) an open and non-punitive atmosphere that encourages reporting of any febrile illness.

SPECIAL ISSUES

Select Agent *R. prowazekii* and *R. rickettsii* are Select Agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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- **Section VIII-E: Viral Agents**

Agent: Hantaviruses

Hantaviruses are negative sense RNA viruses belonging to the genus *Hantavirus* within the family *Bunyaviridae.* The natural hosts of hantaviruses are rodent species and they occur worldwide. Hantavirus pulmonary syndrome (HPS) is a severe disease caused by hantaviruses such as Sin Nombre virus or Andes virus whose hosts are rodents in the subfamily *Sigmodontinae.* This subfamily only occurs in the New World, so HPS is not seen outside North and South America. Hantaviruses in Europe and Asia frequently cause kidney disease, called nephropathica epidemica in Europe, and hemorrhagic fever with renal syndrome (HFRS) in Asia.

Occupational Infections

Documented laboratory-acquired infections have occurred in individuals working with hantaviruses.[1-4] Extreme caution must be used in performing any laboratory operation that may create aerosols (centrigfugation, vortex-mixing, etc.). Rats, voles, and other laboratory rodents, should be conducted with special caution because of the extreme hazard of aerosol infection, especially from infected rodent urine.

Natural Modes of Infection

HPS is a severe, often fatal disease that is caused by Sin Nombre and Andes or related viruses.[5,6] Most cases of human illness have resulted from exposures to naturally infected wild rodents or to their excreta. Person-to-person transmission does not occur, with the exception of a few rare instances documented for Andes virus.[7] Arthropod vectors are not known to transmit hantaviruses.

LABORATORY SAFETY

Laboratory transmission of hantaviruses from rodents to humans via the aerosol route is well documented.[4-7] Exposures to rodent excreta, especially aerosolized infectious urine, fresh necropsy material, and animal bedding are presumed to be associated with risk. Other potential routes of laboratory infection include ingestion, contact of infectious materials with mucous membranes or broken skin and, in particular, animal bites. Viral RNA has been detected in necropsy specimens and in patient blood and plasma obtained early in the course of HPS;[8,9] however, the infectivity of blood or tissues is unknown.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of sera from persons potentially infected with hantaviruses. The use of a certified BSC is recommended for all handling of human body fluids when potential exists for splatter or aerosol.

Potentially infected tissue samples should be handled in BSL-2 facilities following BSL-3 practices and procedures. Cell-culture virus propagation and purification should be carried out in a BSL-3 facility using BSL-3 practices, containment equipment and procedures.

Experimentally infected rodent species known not to excrete the virus can be housed in ABSL-2 facilities using ABSL-2 practices and procedures. Primary physical containment devices including BSCs should be used whenever procedures with potential for generating aerosols are conducted. Serum or tissue samples from potentially infected rodents should be handled at BSL-2 using BSL-3 practices, containment equipment and procedures. All work involving inoculation of virus-containing samples into rodent species permissive for chronic infection should be conducted at ABSL-4.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Agent: Hendra virus (formerly known as Equine Morbillivirus) and Nipah Virus

Hendra virus and *Nipah* virus are members of a newly recognized genus called *Henipavirus,* within the family *Paramyxoviridae.* Outbreaks of a previously unrecognized paramyxovirus, at first called equine morbillivirus, later named Hendra virus, occurred in horses in Australia in 1994 and 1995. During 1998-1999, an outbreak of illness caused by a similar but distinct virus, now known as Nipah virus, occurred in Malaysia and Singapore. Human illness, characterized by fever, severe headache, myalgia and signs of encephalitis occurred in individuals in close contact with pigs (i.e., pig farmers and abattoir workers).[10-14] A few patients developed a respiratory disease. Approximately 40% of patients with encephalitis died. Recently, cases of Nipah virus infection were described in Bangladesh, apparently the result of close contact with infected fruit bats without an intermediate (e.g., pig) host.

Occupational Infections

No laboratory-acquired infections are known to have occurred as a result of Hendra or Nipah virus exposure; however, three people in close contact with ill horses developed encephalitis or respiratory disease and two died.[15-20]

Natural Modes of Infection

The natural reservoir hosts for the Hendra and Nipah viruses appear to be fruit bats of the genus *Pteropus*.[21-23] Studies suggest that a locally occurring member of the genus, *Pteropus giganteus*, is the reservoir for the virus in Bangladesh.[24] Individuals who

had regular contact with bats had no evidence of infection (antibody) in one study in Australia.[25]

LABORATORY SAFETY

The exact mode of transmission of these viruses has not been established. Most clinical cases to date have been associated with close contact with horses, their blood or body fluids (Australia) or pigs (Malaysia/Singapore) but presumed direct transmission from *Pteropus* bats has been recorded in Bangladesh. Hendra and Nipah viruses have been isolated from tissues of infected animals. In the outbreaks in Malaysia and Singapore, viral antigen was found in central nervous system, kidney and lung tissues of fatal human cases[26] and virus was present in secretions of patients, albeit at low levels.[27] Active surveillance for infection of healthcare workers in Malaysia has not detected evidence of occupationally-acquired infections in this setting.[28]

Containment Recommendations

Because of the unknown risks to laboratory workers and the potential impact on indigenous livestock should the virus escape a diagnostic or research laboratory, health officials and laboratory managers should evaluate the need to work with the virus and the containment capability of the facility before undertaking any work with Hendra, Nipah or suspected related viruses. BSL-4 is required for all work with these viruses. Once a diagnosis of Nipah or Hendra virus is suspected, all diagnostic specimens also must be handled at BSL-4. ABSL-4 is required for any work with infected animals.

SPECIAL ISSUES

Select Agent Hendra and Nipah virus are Select Agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Hepatitis A Virus, Hepatitis E Virus

Hepatitis A virus is a positive single-stranded RNA virus, the type species of the *Hepatovirus* genus in the family *Picornaviridae.* Hepatitis E virus is a positive single-stranded RNA virus, the type species of the genus *Hepevirus*, a floating genus not assigned to any family.

Occupational Infections

Laboratory-associated infections with hepatitis A or E viruses do not appear to be an important occupational risk among laboratory personnel. However, hepatitis A is a documented hazard in animal handlers and others working with naturally or experimentally infected chimpanzees and other nonhuman primates.[29] Workers handling other recently captured, susceptible primates (owl monkeys, marmosets) also may be at risk for hepatitis A infection. Hepatitis E virus appears to be less of a risk to personnel than hepatitis A virus, except during pregnancy, when infection can result in severe or fatal disease.

Natural Modes of Infection

Most infections with hepatitis A are foodborne and occasionally water-borne. The virus is present in feces during the prodromal phase of the disease and usually disappears once jaundice occurs. Hepatitis E virus causes acute enterically–transmitted cases of hepatitis, mostly waterborne. In Asia, epidemics involving thousands of cases have occurred.

LABORATORY SAFETY

The agents may be present in feces and blood of infected humans and nonhuman primates. Feces, stool suspensions, and other contaminated materials are the primary hazards to laboratory personnel. Care should be taken to avoid puncture wounds when handling contaminated blood from humans or nonhuman primates. There is no evidence that aerosol exposure results in infection.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for the manipulation of hepatitis A and E virus, infected feces, blood or other tissues. ABSL-2 practices and facilities are recommended for activities using naturally or experimentally-infected nonhuman primates or other animal models that may shed the virus.

Vaccines A licensed inactivated vaccine against hepatitis A is available. Vaccines against hepatitis E are not currently available.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Hepatitis B Virus, Hepatitis C Virus (formerly known as nonA nonB Virus), Hepatitis D Virus

Hepatitis B virus (HBV) is the type species of the *Orthohepadnavirus* genus in the family *Hepadnaviridae*. Hepatitis C virus (HCV) is the type species of the *Hepacivirus* genus in the family *Flaviviridae.* Hepatitis D virus (HDV) is the only member of the genus *Deltavirus.*

These viruses are naturally acquired from a carrier during blood transfusion, vaccination, tattooing, or ear piercing with inadequately sterilized instruments. Non-parenteral routes are also important, and cases may result from domestic and sexual contact, especially homosexual practices.

Individuals who are infected with the HBV are at risk of infection with HDV, a defective RNA virus that requires the presence of HBV virus for replication. Infection with HDV usually exacerbates the symptoms caused by HBV infection.

Occupational Infection

Hepatitis B has been one of the most frequently occurring laboratory-associated infections, and laboratory workers are recognized as a high-risk group for acquiring such infections.[30]

Hepatitis C virus infection can occur in the laboratory situation as well.[31] The prevalence of antibody to hepatitis C (anti-HCV) is slightly higher in medical care workers than in the general population. Epidemiologic evidence indicates that HCV is spread predominantly by the parenteral route.[32]

LABORATORY SAFETY

HBV may be present in blood and blood products of human origin, in urine, semen, CSF and saliva. Parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin are the primary laboratory hazards.[33] The virus may be stable in dried blood or blood components for several days. Attenuated or avirulent strains have not been identified.

HCV has been detected primarily in blood and serum, less frequently in saliva and rarely or not at all in urine or semen. It appears to be relatively unstable to storage at room temperature and repeated freezing and thawing.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. Additional primary containment and personnel precautions, such as those described for BSL-3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. ABSL-2 practices, containment equipment and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other NHP. Gloves should be worn when working with infected animals and when there is the likelihood of skin contact with infectious materials. In addition to these recommended precautions, persons working with HBV, HCV, or other bloodborne pathogens should consult the OSHA

Bloodborne Pathogen Standard.[34] Questions related to interpretation of this Standard should be directed to federal, regional or state OSHA offices.

SPECIAL ISSUES

Vaccines Licensed recombinant vaccines against hepatitis B are available and are highly recommended for and offered to laboratory personnel.[35] Vaccines against hepatitis C and D are not yet available for use in humans, but vaccination against HBV will also prevent HDV infection.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Agent: Human Herpes Virus

The herpesviruses are ubiquitous human pathogens and are commonly present in a variety of clinical materials submitted for virus isolation. Thus far, nine herpesviruses have been isolated from humans: herpes simplex virus-1(HSV-1), HSV-2, human cytomegalovirus (HCMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and human herpesviruses (HHV) 6A, 6B, 7, and 8.[36]

HSV infection is characterized by a localized primary lesion. Primary infection with HSV-1 may be mild and inapparent occurring in early childhood. In approximately 10% of infections overt illness marked by fever and malaise occurs. HSV-1 is a common cause of meningoencephalitis. Genital infections, usually caused by HSV-2, generally occur in adults and are sexually transmissible. Neonatal infections are most frequently caused by HSV-2 but HSV-1 infections are also common. In the neonate, disseminated disease and encephalitis are often fatal. EBV is the cause of infectious mononucleosis. It is also associated the pathogenesis of several lymphomas and nasopharyngeal cancer.[37] EBV is serologically distinct from the other herpesviruses; it infects and transforms B-lymphocytes. HCMV infection is common and often undiagnosed presenting as a nonspecific febrile illness. HCMV causes up to 10% of all cases of mononucleosis in young adults. The most severe form of the disease is seen in infants infected *in utero*. Children surviving infection may evidence mental retardation, microencephaly, motor disabilities and chronic liver disease.[37] HCMV is one of the most common congenital diseases. VZV is the causative agent of chickenpox and herpes zoster. Chickenpox usually occurs in childhood and zoster occurs more commonly in adults. HHV-6 is the causative agent of exanthema subitum (roseola), a common childhood exanthem.[38] Nonspecific febrile illness and febrile seizures are also clinical manifestations of disease. HHV-6 may reactivate in immunocompetent individuals during pregnancy or during critical illness. Two distinct variants, HHV-6A and HHV-6B, exist; the latter causing roseola. HHV-7 is a constitutive inhabitant of adult human saliva.[39] Clinical manifestations are less well understood but the virus has also been associated with roseola. HHV-8, also known as Kaposi's sarcoma-associated virus, was first identified by Chang and co-workers in 1994.[37] HHV-8 is believed to be the causative agent of Kaposi's sarcoma and has been associated with primary effusion lymphoma.[40] The

natural history of HHV-8 has not been completely elucidated. High risk groups for HHV-8 include HIV-infected men who have sex with men and individuals from areas of high endemicity such as Africa or the Mediterranean.[40] The prevalence of HHV-8 is also higher among intravenous drug users than in the general population.[40] At least one report has provided evidence that, in African children, HHV-8 infection may be transmitted from mother to child.[41] While few of the human herpesviruses have been demonstrated to cause laboratory-acquired infections, they are both primary and opportunistic pathogens, especially in immunocompromised hosts. Herpesvirus simiae (B-virus, Monkey B virus) is discussed separately in another agent summary statement in this section.

Natural Modes of Infection

Given the wide array of viruses included in this family, the natural modes of infection vary greatly, as does the pathogenesis of the various viruses. Some have wide host ranges, multiply effectively, and rapidly destroy the cells they infect (HSV-1, HSV-2). Others have restricted host ranges or long replicative cycles (HHV-6).[36] Transmission of human herpesviruses in nature are, in general, associated with contact close, intimate contact with a person excreting the virus in their saliva, urine, or other bodily fluids.[42] VZV is transmitted person-to-person through direct contact, through aerosolized vesicular fluids and respiratory secretions, and indirectly transmitted by fomites. Latency is a trait common to most herpesviruses, although the site and duration vary greatly. For example, EBV will persist in an asymptomatic, latent form in the host immune system, primarily in EBV-specific cytotoxic T cells[37] while latent HSV has been detected only in sensory neurons.[43,44] HHV-8 has been transmitted through organ transplantation[45] and blood transfusion;[46] some evidence suggests non-sexual horizontal transmission.[47]

Occupational Infections

Few of the human herpesviruses have been documented as sources of laboratory acquired infections.

In a limited study, Gartner and co-workers have investigated the HHV-8 immunoglobulin G (IgG) seroprevalence rates for healthcare workers caring for patients with a high risk for HHV-8 infection in a non-endemic area. Healthcare workers in contact with risk group patients were infected more frequently than healthcare workers without contact with risk groups. Workers without contact with risk group patients were infected no more frequently than the control group.[48]

Although this diverse group of indigenous viral agents has not demonstrated a high potential hazard for laboratory-associated infection, frequent presence in clinical materials and common use in research warrant the application of appropriate laboratory containment and safe practices.

LABORATORY SAFETY

Clinical materials and isolates of herpesviruses may pose a risk of infection

following ingestion, accidental parenteral inoculation, and droplet exposure of the mucous membranes of the eyes, nose, or mouth, or inhalation of concentrated aerosolized materials. HHV-8 may be present in human blood or blood products and tissues or saliva. Aerosol transmission cannot be excluded as a potential route of transmission. Clinical specimens containing the more virulent Herpesvirus simiae (B-virus) may be inadvertently submitted for diagnosis of suspected herpes simplex infection. HCMV may pose a special risk during pregnancy because of potential infection of the fetus. All human herpesviruses pose an increased risk to persons who are immunocompromised.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of LAI, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates, or during the necropsy of animals. Primary containment devices (e.g., BSC) should be utilized to prevent exposure of workers to infectious aerosols. Additional containment and procedures, such as those described for BSL-3, should be considered when producing, purifying, and concentrating human herpesviruses, based on risk assessment.

Containment recommendations for herpesvirus simiae (B-virus, Monkey B virus) are described separately in another agent summary statement in this section.

SPECIAL ISSUES

Vaccine A live, attenuated vaccine for varicella zoster is licensed and available in the United States. In the event of a laboratory exposure to a non-immune individual, varicella vaccine is likely to prevent or at least modify disease.[42]

Treatment Antiviral medications are available for treatment of several of the herpesviruses.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Influenza

Influenza is an acute viral disease of the respiratory tract. The most common clinical manifestations are fever, headache, malaise, sore throat and cough. GI tract manifestations (nausea, vomiting and diarrhea) are rare but may accompany the respiratory phase in children. The two most important features of influenza are the

epidemic nature of illness and the mortality that arises from pulmonary complications of the disease.[49]

The influenza viruses are enveloped RNA viruses belonging to the Orthomyxoviridae. There are three serotypes of influenza viruses, A, B and C. Influenza A is further classified into subtypes by the surface glycoproteins that possess either hemagglutinin (H) or neuraminidase (N) activity. Emergence of completely new subtypes (antigenic shift) occurs at irregular intervals with Type A viruses. New subtypes are responsible for pandemics and can result from reassortment of human and avian influenza virus genes. Antigenic changes within a type or subtype (antigenic drift) of A and B viruses are ongoing processes that are responsible for frequent epidemics and regional outbreaks and make the annual reformulation of influenza vaccine necessary.

Influenza viral infections, with different antigenic subtypes, occur naturally in swine, horses, mink, seals and in many domestic and wild avian species. Interspecies transmission and reassortment of influenza A viruses have been reported to occur among, humans and wild and domestic fowl. The human influenza viruses responsible for the 1918, 1957 and 1968 pandemics contained gene segments closely related to those of avian influenza viruses.[50] Swine influenza has also been isolated in human outbreaks.[51]

Control of influenza is a continuing human and veterinary public health concern.

Occupational Infections

LAI have not been routinely documented in the literature, but informal accounts and published reports indicate that such infections are known to have occurred, particularly when new strains showing antigenic shift or drift are introduced into a laboratory for diagnostic/research purposes.[51] Occupationally-acquired, nosocomial infections are documented.[52,53] Laboratory animal-associated infections have not been reported; however, there is possibility of human infection acquired from infected ferrets and vice versa.

Natural Modes of Infection

Airborne spread is the predominant mode of transmission especially in crowded, enclosed spaces. Transmission may also occur through direct contact since influenza viruses may persist for hours on surfaces particularly in the cold and under conditions of low humidity.[50] The incubation period is from one to three days. Recommendations for treatment and prophylaxis of influenza are available.[54]

LABORATORY SAFETY

The agent may be present in respiratory tissues or secretions of humans and most infected animals and birds. In addition, the agent may be present in the intestines and cloacae of many infected avian species. Influenza viruses may be disseminated in multiple organs in some infected animal species. The primary laboratory hazard is

inhalation of virus from aerosols generated by infecting animals or by aspirating, dispensing, mixing, centrifuging or otherwise manipulating virus-infected samples. In addition, laboratory infection can result from direct inoculation of mucus membranes through virus contaminated gloves following handling of tissues, feces or secretions from infected animals. Genetic manipulation has the potential for altering the host range, pathogenicity, and antigenic composition of influenza viruses. The potential for introducing influenza viruses with novel genetic composition into humans is unknown.

Containment Recommendations

BSL-2 facilities, practices and procedures are recommended for diagnostic, research and production activities utilizing contemporary, circulating human influenza strains (e.g., H1/H3/B) and low pathogenicity avian influenza (LPAI) strains (e.g., H1-4, H6, H8-16), and equine and swine influenza viruses. ABSL-2 is appropriate for work with these viruses in animal models. All avian and swine influenza viruses require an APHIS permit. Based on economic ramifications and source of the virus, LPAI H5 and H7 and swine influenza viruses may have additional APHIS permit-driven containment requirements and personnel practices and/or restrictions.

NON-CONTEMPORARY HUMAN INFLUENZA (H2N2) STRAINS

Non-contemporary, wild-type human influenza (H2N2) strains should be handled with increased caution. Important considerations in working with these strains are the number of years since an antigenically related virus last circulated and the potential for presence of a susceptible population. BSL-3 and ABSL-3 practices, procedures and facilities are recommended with rigorous adherence to additional respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators or positive air-purifying respirators (PAPRs) are recommended for use. Cold-adapted, live attenuated H2N2 vaccine strains may continue to be worked with at BSL-2.

1918 INFLUENZA STRAIN

Any research involving reverse genetics of the 1918 influenza strain should proceed with *extreme* caution. The risk to laboratory workers is unknown at the present time, but the pandemic potential is thought to be significant. Until further risk assessment data are available, the following practices and conditions are recommended for manipulation of reconstructed 1918 influenza viruses and laboratory animals infected with the viruses. These practices and procedures are considered minimum standards for work with the fully reconstructed virus.

- BSL-3 and ABSL-3 practices, procedures and facilities.
- Large laboratory animals such as NHP should be housed in primary barrier systems in ABSL-3 facilities.

- Rigorous adherence to additional respiratory protection and clothing change protocols.
- Use of negative pressure, HEPA-filtered respirators or PAPRs.
- Use of HEPA filtration for treatment of exhaust air.
- Amendment of personnel practices to include personal showers prior to exiting the laboratory.

HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI)

Manipulating HPAI viruses in biomedical research laboratories requires similar caution because some strains may pose increased risk to laboratory workers and have significant agricultural and economic implications. BSL-3 and ABSL-3 practices, procedures and facilities are recommended along with clothing change and personal showering protocols. Loose-housed animals infected with HPAI strains must be contained within BSL-3-Ag facilities (See Appendix D). Negative pressure, HEPA-filtered respirators or positive air-purifying respirators are recommended for HPAI viruses with potential to infect humans. The HPAI are agricultural Select Agents requiring registration of personnel and facilities with the lead agency for the institution (CDC or USDA-APHIS). An APHIS permit is also required. Additional containment requirements and personnel practices and/or restrictions may be added as conditions of the permit.

OTHER INFLUENZA RECOMBINANT OR REASSORTANT VIRUSES

When considering the biocontainment level and attendant practices and procedures for work with other influenza recombinant or reassortant viruses, the local IBC should consider but not limit consideration to the following in the conduct of protocol-driven risk assessment.

- The gene constellation used.
- Clear evidence of reduced virus replication in the respiratory tract of appropriate animal models, compared with the level of replication of the wild-type parent virus from which it was derived.
- Evidence of clonal purity and phenotypic stability.
- The number of years since a virus that was antigenically related to the donor of the hemagglutinin and neuraminidase genes last circulated.

If adequate risk assessment data are not available, a more cautious approach utilizing elevated biocontainment levels and practices is warranted. There may be specific

requirements regarding the setting of containment levels if your institution is subject to the *NIH Guidelines*.

SPECIAL ISSUES

Occupational Health Considerations

Institutions performing work with HPAI and avian viruses that have infected humans; non-contemporary wild-type human influenza strains, including recombinants and reassortants; and viruses created by reverse genetics of the 1918 pandemic strain should develop and implement a specific medical surveillance and response plan. At the minimum these plans should 1) require storage of baseline serum samples from individuals working with these influenza strains; 2) strongly recommend annual vaccination with the currently licensed influenza vaccine for such individuals; 3) provide employee counseling regarding disease symptoms including fever, conjunctivitis and respiratory symptoms; 4) establish a protocol for monitoring personnel for these symptoms; and 5) establish a clear medical protocol for responding to suspected laboratory-acquired infections. Antiviral drugs (e.g., oseltamivir, amantadine, rimantadine, zanamivir) should be available for treatment and prophylaxis, as necessary.[54] It is recommended that the sensitivities of the virus being studied to the antivirals be ascertained. All personnel should be enrolled in an appropriately constituted respiratory protection program.

Influenza viruses may require USDA and/or USPHS import permits depending on the host range and pathogenicity of the virus in question.

Select Agent Influenza virus is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis (LCM) is a rodent-borne viral infectious disease that presents as aseptic meningitis, encephalitis, or meningoencephalitis. The causative agent is the LCM virus (LCMV) that was initially isolated in 1933. The virus is the protypical member of the family *Arenaviridae.*

Occupational Infections

LAI with LCM virus are well documented. Most infections occur when chronic viral infection exists in laboratory rodents, especially mice, hamsters and guinea pigs.[55-57]

Nude and severe combined immune deficient (SCID) mice may pose a special risk of harboring silent chronic infections. Inadvertently infected cell cultures also represent a potential source of infection and dissemination of the agent.

Natural Modes of Infection

LCM and milder LCMV infections have been reported in Europe, the Americas, Australia, and Japan, and may occur wherever infected rodent hosts of the virus are found. Several serologic studies conducted in urban areas have shown that the prevalence of LCMV infection among humans ranges from 2% to 10%. Seroprevalence of 37.5% has been reported in humans in the Slovak Republic.[58]

The common house mouse, *Mus musculus*, naturally spreads LCMV. Once infected, these mice can become chronically infected as demonstrated by the presence of virus in blood and/or by persistently shedding virus in urine. Infections have also occurred in NHP in zoos, including macaques and marmosets (*Callitrichid* hepatitis virus is a LCMV).

Humans become infected by inhaling infectious aerosolized particles of rodent urine, feces, or saliva, by ingesting food contaminated with virus; by contamination of mucous membranes with infected body fluids; or by directly exposing cuts or other open wounds to virus-infected blood. Four recipients of organs from a donor who had unrecognized disseminated LCMV infection sustained severe disease and three succumbed. The source of donor infection was traced to a pet hamster that was not overtly ill.[59]

LABORATORY SAFETY

The agent may be present in blood, CSF, urine, secretions of the nasopharynx, feces and tissues of infected animal hosts and humans. Parenteral inoculation, inhalation, contamination of mucous membranes or broken skin with infectious tissues or fluids from infected animals are common hazards. Aerosol transmission is well documented.[55]

Of special note, tumors may acquire LCMV as an adventitious virus without obvious effects on the tumor. Virus may survive freezing and storage in liquid nitrogen for long periods. When infected tumor cells are transplanted, subsequent infection of the host and virus excretion may ensue. Pregnant women infected with LCMV have transmitted the virus to their fetus with death or serious central nervous system malformation as a consequence.[60]

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are suitable for activities utilizing known or potentially infectious body fluids, and for cell culture passage of laboratory-adapted strains. BSL-3 is required for activities with high potential for aerosol production, work with production quantities or high concentrations of infectious

materials, and for manipulation of infected transplantable tumors, field isolates and clinical materials from human cases. Strains of LCMV that are shown to be lethal in non-human primates should be handled at BSL-3. ABSL-2 practices, containment equipment, and facilities are suitable for studies in adult mice with strains requiring BSL-2 containment. Work with infected hamsters also should be done at ABSL-3.

SPECIAL ISSUES

Vaccines Vaccines are not available for use in humans.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Poliovirus

Poliovirus is the type species of the *Enterovirus* genus in the family *Picornaviridae.* Enteroviruses are transient inhabitants of the gastrointestinal tract, and are stable at acid pH. Picornaviruses are small, ether-insensitive viruses with an RNA genome.

There are three poliovirus serotypes (P1, P2, and P3). Immunity to one serotype does not produce significant immunity to the other serotypes.

Occupational Infections

Laboratory-associated poliomyelitis is uncommon. Twelve cases, including two deaths, were reported between 1941 and 1976.[57,61] No laboratory-associated poliomyelitis has been reported for nearly 30 years. Both inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV) are highly effective in preventing disease, but neither vaccine provides complete protection against infection. Poliovirus infections among immunized laboratory workers are uncommon but remain undetermined in the absence of laboratory confirmation. An immunized laboratory worker may unknowingly be a source of poliovirus transmission to unvaccinated persons in the community.[62]

Natural Modes of Infection

At one time poliovirus infection occurred throughout the world. Transmission of wild poliovirus ceased in the United States in 1979, or possibly earlier. A polio eradication program conducted by the Pan American Health Organization led to elimination of polio from the Western Hemisphere in 1991. The Global Polio Eradication Program has dramatically reduced poliovirus transmission throughout the world.

Humans are the only known reservoir of poliovirus, which is transmitted most frequently by persons with inapparent infections. Person-to-person spread of poliovirus

via the fecal-oral route is the most important route of transmission, although the oral-oral route may account for some cases.

LABORATORY SAFETY

The agent is present in the feces and in throat secretions of infected persons and in lymph nodes, brain tissue, and spinal cord tissue in fatal cases. For non-immunized persons in the laboratory, ingestion or parenteral inoculation are the primary routes of infection. For immunized persons, the primary risks are the same, except for parenteral inoculation, which likely presents a lower risk. The importance of aerosol exposure is unknown. Laboratory animal-associated infections have not been reported, but infected nonhuman primates should be considered to present a risk.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing wild poliovirus infectious culture fluids, environmental samples, and clinical materials, in addition, potentially infectious materials collected for any purpose should be handled at BSL-2. Laboratory personnel working with such materials must have documented polio vaccination. Persons who have had a primary series of OPV or IPV and who are at an increased risk can receive another dose of IPV, but available data do not indicate the need for more than a single lifetime IPV booster dose for adults. [63] ABSL-2 practices, containment equipment, and facilities are recommended for studies of virulent viruses in animals. Laboratories should use authentic Sabin OPV attenuated strains unless there are strong scientific reasons for working with wild polioviruses.

In anticipation of polio eradication, the WHO recommends destruction of all poliovirus stocks and potential infectious materials if there is no longer a programmatic or research need for such materials.[64] Institutions/laboratories in the United States that currently retain wild poliovirus infectious or potential infectious material should be on the United States National Inventory maintained by CDC. When one year has elapsed after detection of the last wild poliovirus worldwide, CDC will inform relevant institutions/laboratories about additional containment procedures. Safety recommendations are subject to change based on international polio eradication activities.

SPECIAL ISSUES

When OPV immunization stops, global control and biosafety requirements for wild as well as attenuated (Sabin) poliovirus materials are expected to become more stringent, consistent with the increased consequences of inadvertent transmission to a growing susceptible community.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Poxviruses

Four genera of the subfamily *Chordopoxvirinae*, family *Poxviridae*, (*Orthopoxvirus*, *Parapoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus*) contain species that can cause lesions on human skin or mucous membranes with mild to severe systemic rash illness in laboratorians. Species within the first three genera mostly arise as zoonotic agents.[65,66] Laboratory-acquired poxvirus infections of most concern are from the orthopoxviruses that infect humans: variola virus (causes smallpox; human-specific), monkeypox virus (causes smallpox-like disease, *cowpox virus* (causes skin pustule, generalized rash), and vaccinia virus (causes skin pustule, systemic illness).[65-70]

Occupational Infections

Vaccinia virus, the leading agent of laboratory-acquired poxvirus infections, is used to make the current smallpox vaccine and may occur as a rare zoonosis.[65,66] Laboratory-acquired infections with standard, mutant, or bioengineered forms of vaccinia virus have occurred, even in previously vaccinated laboratorians. In addition, vaccination with live vaccinia virus sometimes has side effects, which range from mild events (e.g., fever, fatigue, swollen lymph nodes) to rare, severe, and at times fatal outcomes (e.g., generalized vaccinia, encephalitis, vaccinia necrosum, eczema vaccinatum, ocular keratitis, corneal infection, fetal infection of pregnancy, and possibly myocardial infarction, myopericarditis, or angina), thus vaccination contraindications should be carefully followed.[65,68-70]

Natural Modes of Infection

Smallpox has been eradicated from the world since 1980, but monkey pox virus is endemic in rodents in parts of Africa. Importation of African rodents into North America in 2003 resulted in an outbreak of monkeypox in humans.[67] Molluscum contagiosum, a disease due to *Molluscipoxvirus* infection, results in pearly white lesions that may persist for months in persons immunocompromised for various reasons, including chronic illness, AIDS, other infections, medications, cancer and cancer therapies, or pregnancy.[65]

LABORATORY SAFETY

Poxviruses are stable in a wide range of environmental temperatures and humidity and may be transmitted by fomites.[65] Virus may enter the body through mucous membranes, broken skin, or by ingestion, parenteral inoculation or droplet or fine-particle aerosol inhalation. Sources of laboratory-acquired infection include exposure to aerosols, environmental samples, naturally or experimentally infected animals, infectious cultures, or clinical samples, including vesiculopustular rash lesion fluid or crusted scabs, various tissue specimens, excretions and respiratory secretions.

Containment Recommendations

Worldwide, all live variola virus work is to be done only within WHO approved BSL-4/ABSL-4 facilities; one is at the CDC in Atlanta and the other is at the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia.[71]

In general, all persons working in or entering laboratory or animal care areas where activities with vaccinia, monkey pox, or cow pox viruses are being conducted should have evidence of satisfactory vaccination. Vaccination is advised every three years for work with monkeypox virus and every 10 years for cowpox and vaccinia viruses (neither vaccination nor vaccinia immunoglobulin protect against poxviruses of other genera).[68-70] Vaccination is not required for individuals working only in laboratories where no other orthpoxviruses or recombinants are handled.[70]

ABSL-3 practices, containment equipment, and facilities are recommended for monkeypox work in experimentally or naturally infected animals. BSL-2 facilities with BSL-3 practices are advised if other work with monkeypox virus by vaccinated personnel. These practices include the use of Class-I or -II BSCs and barriers, such as safety cups or sealed rotors, for all centrifugations. The *NIH Guidelines* have assessed the risk of manipulating attenuated vaccinia strains (modified virus Ankara (MVA), NYVAC, TROVAC, and ALVAC) in areas where no other human orthopoxviruses are being used and have recommended BSL-1.[71] However, higher levels of containment are recommended if these strains are used in work areas where other orthopoxviruses are manipulated. BSL-2 and ABSL-2 plus vaccination, are recommended for work with most other poxviruses.

SPECIAL ISSUES

Other Considerations

The CDC internet site www.cdc.gov provides information on poxviruses, especially variola and monkeypox viruses, and on smallpox vaccination, and for reporting vaccination adverse events. Clinical and other laboratories using poxviruses and clinicians can phone the CDC Clinician Information Line (877-554-4625) and/or the CDC public information hotline (888-246-2675) concerning variola and other human poxvirus infections, smallpox vaccine, vaccinia immunoglobulin, poxvirus antiviral drugs, or other treatments or quarantine issues. Contact CDC regarding applications to transfer monkeypox viruses.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Rabies Virus (and related lyssaviruses)

Rabies is an acute, progressive, fatal encephalitis caused by negative-stranded RNA viruses in the genus *Lyssavirus*, family *Rhabdoviridae*.[72] *Rabies virus* is the

representative member (type species) of the genus. Members of the group include Australian bat lyssavirus, Duvenhage virus, European bat lyssavirus 1, European bat lyssavirus 2, Lagos bat virus, and Mokola virus.

Occupational Infections

LAI are extremely rare; two have been documented. Both resulted from presumed exposure to high concentrations of infectious aerosols, one generated in a vaccine production facility,[73] and the other in a research facility.[74] Naturally or experimentally infected animals, their tissues, and their excretions are a potential source of exposure for laboratory and animal care personnel.

Natural Modes of Infection

The natural hosts of rabies are many bat species and terrestrial carnivores, but most mammals can be infected. The saliva of infected animals is highly infectious, and bites are the usual means of transmission, although infection through superficial skin lesions or mucosa is possible.

LABORATORY SAFETY

When working with infected animals, the highest viral concentrations are present in central nervous system (CNS) tissue, salivary glands, and saliva, but rabies viral antigens may be detected in all innervated tissues. Accidental parenteral inoculation, cuts, or needle sticks with contaminated laboratory equipment, bites by infected animals, and exposure of mucous membranes or broken skin to infectious tissue or fluids, are the most likely sources for exposure of laboratory and animal care personnel. Infectious aerosols have not been a demonstrated hazard to personnel working with routine clinical materials and conducting diagnostic examinations. Fixed and attenuated strains of virus are presumed to be less hazardous, but the two recorded cases of laboratory-associated rabies resulted from presumed exposure to the fixed Challenge Virus Standard and Street Alabama Dufferin strains, respectively.

Containment Recommendations

BSL-2 and/or ABSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials or animals. Pre-exposure rabies vaccination is recommended for all individuals prior to working with lyssaviruses or infected animals, or engaging in diagnostic, production, or research activities with these viruses.[75] Rabies vaccination also is recommended for all individuals entering or working in the same room where lyssaviruses or infected animals are used. Prompt administration of postexposure booster vaccinations is recommended following recognized exposures in previously vaccinated individuals per current guidelines.[76] For routine diagnostic activities, it is not always feasible to open the skull or remove the brain of an infected animal within a BSC, but it is pertinent to use appropriate methods and personal protection equipment, including dedicated laboratory clothing,

heavy protective gloves to avoid cuts or sticks from cutting instruments or bone fragments, and a face shield or PAPR to protect the skin and mucous membranes of the eyes, nose, and mouth from exposure to tissue fragments or infectious droplets.

If a Stryker saw is used to open the skull, avoid contacting brain tissue with the blade of the saw. Additional primary containment and personnel precautions, such as those described for BSL-3, are indicated for activities with a high potential for droplet or aerosol production, and for activities involving large production quantities or high concentrations of infectious materials.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Retroviruses, Including Human and Simian Immunodeficiency Viruses (HIV and SIV)

The family *Retroviridae* is divided into two subfamilies, the *Orthoretrovirinae* with six genera including the *Lentivirus* genus which includes HIV-1 and HIV-2. Other important human pathogens are human T-lymphotropic viruses 1 and 2 (HTLV-1 and HTLV-2), members of the *Deltaretrovirus* genus. And the *Spumaretrovirinae* with one genus *Spumavirus* containing a variety of NHP viruses (foamy viruses) which can occasionally infect humans in close contact with NHP.

Occupational Infections

Data on occupational HIV transmission in laboratory workers are collected through two CDC-supported national surveillance systems: surveillance for 1) AIDS and 2) HIV-infected persons who may have acquired their infection through occupational exposures. For surveillance purposes, laboratory workers are defined as those persons, including students and trainees, who have worked in a clinical or HIV laboratory setting anytime since 1978. Cases reported in these two systems are classified as either documented or possible occupational transmission. Those classified as documented occupational transmission had evidence of HIV seroconversion (a negative HIV-antibody test at the time of the exposure which converted to positive) following a discrete percutaneous or mucocutaneous occupational exposure to blood, body fluids, or other clinical or laboratory specimens. As of June 1998, CDC had reports of 16 laboratory workers (all clinical) in the United States with documented occupational transmission.[77]

Workers have been reported to develop antibodies to simian immunodeficiency virus (SIV) following exposures. One case was associated with a needle-stick that occurred while the worker was manipulating a blood-contaminated needle after bleeding an SIV-infected macaque monkey.[78] Another case involved a laboratory worker who

handled macaque SIV-infected blood specimens without gloves. Though no specific incident was recalled, this worker had dermatitis on the forearms and hands while working with the infected blood specimens.[79] A third worker[80] was exposed to SIV-infected primate blood through a needle-stick and subsequently developed antibodies to SIV. To date there is no evidence of illness or immunological incompetence in any of these workers.

Natural Modes of Infection

Retroviruses are widely distributed as infectious agents of vertebrates. Within the human population spread is by close sexual contact or parenteral exposure through blood or blood products.

LABORATORY SAFETY

HIV has been isolated from blood, semen, saliva, tears, urine, CSF, amniotic fluid, breast milk, cervical secretion, and tissue of infected persons and experimentally infected nonhuman primates.[81]

Although the risk of occupationally acquired HIV is primarily through exposure to infected blood, it is also prudent to wear gloves when manipulating other body fluids such as feces, saliva, urine, tears, sweat, vomitus, and human breast milk. This also reduces the potential for exposure to other microorganisms that may cause other types of infections.

In the laboratory, virus should be presumed to be present in all blood or clinical specimens contaminated with blood, in any unfixed tissue or organ (other than intact skin) from a human (living or dead), in HIV cultures, in all materials derived from HIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

SIV has been isolated from blood, CSF, and a variety of tissues of infected nonhuman primates. Limited data exist on the concentration of virus in semen, saliva, cervical secretions, urine, breast milk, and amniotic fluid. Virus should be presumed to be present in all SIV cultures, in animals experimentally infected or inoculated with SIV, in all materials derived from SIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.[82]

The skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, and mouth should be considered as potential pathways for entry of these retroviruses during laboratory activities. Whether infection can occur via the respiratory tract is unknown. The need for using sharps in the laboratory should be evaluated. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other potentially infected materials.[80]

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities involving blood-contaminated clinical specimens, body fluids and tissues. HTLV-1 and HTLV-2 should also be handled at this level. Activities such as producing research-laboratory-scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a BSL-2 facility, using BSL-3 practices. Activities involving large-scale volumes or preparation of concentrated HIV or SIV are conducted at BSL-3. ABSL-2 is appropriate for NHP and other animals infected with HIV or SIV. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2.

In addition to the aforementioned recommendations, persons working with HIV, SIV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.[83] Questions related to interpretation of this standard should be directed to federal, regional or state OSHA offices.

SPECIAL ISSUES

It is recommended that all institutions establish written policies regarding the management of laboratory exposure to HIV and SIV; including treatment and prophylaxis protocols (See Chapter 7).

The risk associated with retroviral vector systems can vary significantly; especially lentiviral vectors. Because each gene transfer system can vary significantly, no specific guideline can be offered other than to have all gene transfer protocols reviewed by an IBC.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: SARS coronavirus

Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a previously undescribed coronavirus, SARS-associated coronavirus (SARS-CoV) within the family *Coronaviridae.* SARS was retrospectively recognized in China in November 2002. Over the next few months, the illness spread to other south-east Asian countries, North America, South America, and Europe following major airline routes. The majority of disease spread occurred in hospitals, among family members and contacts of hospital workers. From November 2002 through July 2003, when the global outbreak was contained, a total of 8,098 probable cases of SARS were reported to the WHO from 29 countries.[84]

In general, SARS patients present with fever (temperature greater than 100.4°F [>38.0°C]), malaise and myalgias quickly followed by respiratory symptoms including shortness of breath and cough. Ten to 20 percent of patients may have diarrhea. Review of probable cases indicates that the shortness of breath sometimes rapidly progresses to respiratory failure requiring ventilation. The case fatality rate is about 11%.

Occupational Infections

Healthcare workers are at increased risk of acquiring SARS from an infected patient especially if involved in pulmonary/respiratory procedures such as endotracheal intubation, aerosolization or nebulization of medications, diagnostic sputum induction, airway suctioning, positive pressure ventilation and high-frequency oscillatory ventilation.

Two confirmed episodes of SARS-CoV transmission to laboratory workers occurred in research laboratories in Singapore and Taiwan.[84,85] Both occurrences were linked to breaches in laboratory practices. Laboratory-acquired infections in China, during 2004, demonstrated secondary and tertiary spread of the disease to close contacts and healthcare providers of one of the employees involved.[86] Although, to date, no laboratory-acquired cases have been associated with the routine processing of diagnostic specimens, SARS coronavirus represents an emerging infectious disease for which risk to the medical and laboratory community is not fully understood.

Natural Modes of Infection

The mode of transmission in nature is not well understood. It appears that SARS is transmitted from person to person through close contact such as caring for, living with, or having direct contact with respiratory secretions or body fluids of a suspect or probable case.[87] SARS is thought to be spread primarily through droplets, aerosols and possibly fomites. The natural reservoir for SARS CoV is unknown at this time.

LABORATORY SAFETY

SARS-CoV may be detected in respiratory, blood, or stool specimens. The exact mode of transmission of SARS-CoV laboratory-acquired infection has not been established, but in clinical settings, the primary mode of transmission appears through direct or indirect contact of mucous membranes with infectious respiratory droplets.[88,89]

Containment Recommendations

In clinical laboratories, whole blood, serum, plasma and urine specimens should be handled using Standard Precautions which includes use of gloves, gown, mask, and eye protection. Any procedure with the potential to generate aerosols (e.g., vortexing or sonication of specimens in an open tube) should be performed in a BSC. Use sealed centrifuge rotors or gasketed safety carriers for centrifugation. Rotors and safety carriers

should be loaded and unloaded in a BSC. Procedures conducted outside a BSC must be performed in a manner that minimizes the risk of personnel exposure and environmental release.

The following procedures may be conducted in the BSL-2 setting: pathologic examination and processing of formalin-fixed or otherwise inactivated tissues, molecular analysis of extracted nucleic acid preparations, electron microscopic studies with glutaraldehyde-fixed grids, routine examination of bacterial and fungal cultures, routine staining and microscopic analysis of fixed smears, and final packaging of specimens for transport to diagnostic laboratories for additional testing (specimens should already be in a sealed, decontaminated primary container).

Activities involving manipulation of untreated specimens should be performed in BSL-2 facilities following BSL-3 practices. In the rare event that a procedure or process involving untreated specimens cannot be conducted in a BSC, gloves, gown, eye protection, and respiratory protection (acceptable methods of respiratory protection include: a properly fit-tested, National Institute for Occupational Safety and Health (NIOSH)-approved filter respirator (N-95 or higher level) or a PAPR equipped with HEPA filters) should be used. All personnel that may use respiratory protective devices should be enrolled in an appropriately constituted respiratory protection program.

Work surfaces should be decontaminated upon completion of work with appropriate disinfectants. All waste must be decontaminated prior to disposal.

SARS-CoV propagation in cell culture and the initial characterization of viral agents recovered in cultures of SARS specimens must be performed in a BSL-3 facility using BSL-3 practices and procedures. Risk assessment may dictate the additional use of respiratory protection.

Inoculation of animals for potential recovery of SARS-CoV from SARS samples, research studies and protocols involving animal inoculation for characterization of putative SARS agents must be performed in ABSL-3 facilities using ABSL-3 work practices. Respiratory protection should be used as warranted by risk assessment.

In the event of any break in laboratory procedure or accidents (e.g. accidental spillage of material suspected of containing SARS-CoV), procedures for emergency exposure management and/or environmental decontamination should be immediately implemented and the supervisor should be notified. The worker and the supervisor, in consultation with occupational health or infection control personnel, should evaluate the break in procedure to determine if an exposure occurred (See Special Issues, below).

SPECIAL ISSUES

Occupational Health Considerations

Institutions performing work with SARS coronavirus should require storage of a baseline serum sample from individuals who work with the virus or virus-containing specimens. Personnel working with the virus or samples containing or potentially containing the virus should be trained regarding the symptoms of SARS-CoV infection and counseled to report any fever or respiratory symptoms to their supervisor immediately. They should be evaluated for possible exposure and the clinical features and course of their illness should be closely monitored. Institutions performing work with the SARS-CoV or handling specimens likely to contain the agent should develop and implement a specific occupational medical plan with respect to this agent. The plan, at a minimum, should contain procedures for managing:

- identifiable breaks in laboratory procedures;
- exposed workers without symptoms;
- exposed workers who develop symptoms within ten days of an exposure.;
- symptomatic laboratory workers with no recognized exposure.

Further information and guidance regarding the development of a personnel exposure response plan is available from the CDC.[90] Laboratory workers who are believed to have had a laboratory exposure to SARS-CoV should be evaluated, counseled about the risk of SARS-CoV transmission to others, and monitored for fever or lower respiratory symptoms as well as for any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, and diarrhea.

Local and/or state public health departments should be promptly notified of laboratory exposures and illness in exposed laboratory workers.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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• Section VIII-F: Arboviruses and Related Zoonotic Viruses

In 1979, the American Committee on Arthropod-Borne Viruses (ACAV) Subcommittee on Arbovirus Laboratory Safety (SALS) first provided biosafety recommendations for each of the 424 viruses then registered in the International Catalogue of Arboviruses, including Certain Other Viruses of Vertebrates.[1] Working together, SALS, the CDC and the NIH have periodically updated the catalogue by providing recommended biosafety practices and containment for arboviruses registered since 1979. These recommendations are based, in part, on risk assessments derived from information provided by a worldwide survey of laboratories working with arboviruses, new published reports on the viruses, as well as discussions with scientists working with each virus.

Table 1, located at the end of this section, provides an alphabetical listing of 597 viruses and includes common name, virus family or genus, acronym, BSL recommendation, the basis for the rating, the antigenic group[2] (if known), HEPA filtration requirements, and regulatory requirements (i.e. import/export permits from either the CDC or the USDA). In addition, many of the organisms are classified as Select Agents and require special security measures to possess, use, or transport (See Appendix F). Table 2 provides a key for the SALS basis for assignment of viruses listed in Table 1.

Agent summary statements have been included for certain arboviruses. They were submitted by a panel of experts for more detailed consideration due to one or more of the following factors:

- at the time of writing this edition the organism represented an emerging public health threat in the United States;
- the organism presented unique biocontainment challenge(s) that required further detail;
- the organism presented a significant risk of laboratory-acquired infection.

These recommendations were made in August 2005; requirements for biosafety, shipping, and select agent registration can change. Please be sure to confirm the requirements with the appropriate Federal agency. If the pathogen of interest is one listed in Appendix D, contact the USDA for additional biosafety requirements. USDA guidance may supersede the information found in this chapter.

Recommendations for the containment of infected arthropod vectors were drafted by a subcommittee of the American Committee on Medical Entomology (ACME), and circulated widely among medical entomology professionals (See Appendix E).

Some commonly used vaccine strains for which attenuation has been firmly established are recognized by SALS. These vaccine strains may be handled safely at BSL-2 (Table 3). The agents in Table 2 may require permits from USDA/DOC/DHHS.

Table 2

EXPLANATION OF SYMBOLS USED IN TABLE 2 TO DEFINE BASIS FOR ASSIGNMENT OF VIRUSES TO BIOSAFETY LEVELS

SYMBOL	DEFINITION
S	Results of SALS survey and information from the Catalog.[1]
IE	Insufficient experience with virus in laboratory facilities with low biocontainment.
A	Additional criteria.
A1	Disease in sheep, cattle or horses.
A2	Fatal human laboratory infection – probably aerosol.
A3	Extensive laboratory experience and mild nature of aerosol laboratory infections justifies BSL-2.
A4	Placed in BSL-4 based on the close antigenic relationship with a known BSL-4 agent plus insufficient experience.
A5	BSL-2 arenaviruses are not known to cause serious acute disease in humans and are not acutely pathogenic for laboratory animals including primates. In view of reported high frequency of laboratory aerosol infection in workers manipulating high concentrations of Pichinde virus, it is strongly recommended that work with high concentrations of BSL-2 arenaviruses be done at BSL-3.
A6	Level assigned to prototype or wild-type virus. A lower level may be recommended for variants with well-defined reduced virulence characteristics.
A7	Placed at this biosafety level based on close antigenic or genetic relationship to other viruses in a group of 3 or more viruses, all of which are classified at this level.
A8	BSL-2 hantaviruses are not known to cause laboratory infections, overt disease in humans, or severe disease in experimental primates. Because of antigenic and biologic relationships to highly pathogenic hantaviruses and the likelihood that experimentally infected rodents may shed large amounts of virus, it is recommended that work with high concentrations or experimentally infected rodents be conducted at BSL-3.

Table 3

VACCINE STRAINS OF BSL-3 AND -4 VIRUSES THAT MAY BE HANDLED AS BSL-2

VIRUS	VACCINE STRAIN
Chikungunya	181/25
Junin	Candid #1
Rift Valley fever	MP-12
Venezuelan equine encephalomyelitis	TC83
Yellow fever	17-D
Japanese encephalitis	14-14-2

Based on the recommendations listed within Tables 2 and 3, the following guidelines should be adhered to where applicable.

VIRUSES WITH BSL-2 CONTAINMENT RECOMMENDED

The recommendation for conducting work with the viruses listed in Table 1 at BSL-2 was based on the existence of adequate historical laboratory experience to assess the risks when working with this group of viruses. This indicates a) no overt laboratory-associated infections are reported, b) infections resulted from exposures other than by infectious aerosols, or c) if disease from aerosol exposure is documented, it is uncommon.

Laboratory Hazards

Agents listed in this group may be present in blood, CSF, various tissues, and/or infected arthropods, depending on the agent and the stage of infection. The primary laboratory hazards comprise accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites of infected laboratory rodents or arthropods. Properly maintained BSCs, preferable Class II, or other appropriate personal protective equipment or physical containment devices are used whenever procedure with a potential for creating infectious aerosols or splashes are conducted.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities with potentially infectious clinical materials and arthropods and for manipulations of infected tissue cultures, embryonate hen’s eggs, and rodents.

Large quantities and/or high concentrations of any virus have the potential to overwhelm both innate immune mechanisms and vaccine-induced immunity. When a BSL-2 virus is being produced in large quantities or in high concentrations, additional risk assessment is required. This might indicate BSL-3 practices, including additional respiratory protection, based on the risk assessment of the proposed experiment.

VIRUSES WITH BSL-3 CONTAINMENT RECOMMENDED

The recommendations for viruses listed in Table 2 that require BSL-3 containment are based on multiple criteria. SALS considered the laboratory experience for some viruses to be inadequate to assess risk, regardless of the available information regarding disease severity. In some cases, SALS recorded overt LAI transmitted by the aerosol route in the absence or non-use of protective vaccines, and considered that the natural disease in humans is potentially severe, life threatening, or causes residual damage.[1] Arboviruses also were classified as requiring BSL-3 containment if they caused diseases in domestic animals in countries outside of the United States.

Laboratory Hazards

The agents listed in this group may be present in blood, CSF, urine, and exudates, depending on the specific agent and stage of disease. The primary laboratory hazards are exposure to aerosols of infectious solutions and animal bedding, accidental parenteral inoculation, and contact with broken skin. Some of these agents (e.g., VEE virus) may be relatively stable in dried blood or exudates.

Containment Recommendations

BSL-3 practices, containment equipment, and facilities are recommended for activities using potentially infectious clinical materials and infected tissue cultures, animals, or arthropods.

A licensed attenuated live virus is available for immunization against yellow fever. It is recommended for all personnel who work with this agent or with infected animals, and those entering rooms where the agents or infected animals are present.

Junin virus has been reclassified to BSL-3, provided that all at-risk personnel are immunized and the laboratory is equipped with HEPA-filtered exhaust. SALS also has reclassified Central European tick-borne encephalitis (CETBE) viruses to BSL-3, provided all at-risk personnel are immunized. CETBE is not a registered name in *The International Catalogue of Arboviruses* (1985). Until the registration issue is resolved taxonomically, CETBE refers to the following group of very closely related, if not essentially identical, tick-borne flaviviruses isolated from Czechoslovakia, Finland and Russia: Absettarov, Hanzalova, Hypr, and Kumlinge viruses. While there is a vaccine available that confers immunity to the CETBE group of genetically (>98%) homogeneous viruses, the efficacy of this vaccine against Russian spring-summer encephalitis (RSSE) virus infections has not been established. Thus, the CETBE group of viruses has been reclassified as BSL-3 when personnel are immunized with CETBE vaccine, while RSSE remains classified as BSL-4. It should be noted that CETBE viruses are currently listed as Select Agents and require special security and permitting considerations (See Appendix F).

Investigational vaccines for eastern equine encephalomyelitis (EEE) virus, Venezuelan Equine Encephalitis (VEE), western equine encephalomyelitis (WEE) virus, and Rift Valley fever viruses (RVFV), may be available in limited quantities and

administered on-site at the Special Immunization Program, USAMRIID. Details are available at the end of this section.

The use of investigational vaccines for laboratory personnel should be considered if the vaccine is available, initial studies have shown the vaccine to be effective in producing an appropriate immunologic response, and the adverse effects of vaccination are within acceptable parameters. The decision to recommend vaccines for laboratory personnel must be carefully considered and based on an risk assessment which includes a review of the characteristics of the agent and the disease, benefits versus the risk of vaccination, the experience of the laboratory personnel, laboratory procedures to be used with the agent, and the contraindications for vaccination including the health status of the employee.

If the investigational vaccine is contraindicated, does not provide acceptable reliability for producing an immune response, or laboratory personnel refuse vaccination, the use of appropriate personal protective equipment may provide an alternative. Respiratory protection, such as use of a PAPR, should be considered in areas using organisms with a well-established risk of aerosol infections in the laboratory, such as VEE viruses. Any respiratory protection equipment must be provided in accordance with the institution's respiratory protection program. Other degrees of respiratory protection may be warranted based on an assessment of risk as defined in Chapter 2 of this manual. All personnel in a laboratory with the infectious agent must use comparable personal protective equipment that meets or exceeds the requirements, even if they are not working with the organism. Sharps precautions as described under BSL-2 and BSL-3 requirements must be continually and strictly reinforced, regardless of whether investigational vaccines are used.

Non-licensed vaccines are available in limited quantities and administered on-site at the Special Immunization Program of USAMRIID, located at Ft. Detrick, Frederick MD. As IND vaccines are administered under a cooperative agreement between the U.S. Army and the individual's requesting organization. Contact the Special Immunization Program by telephone at (301) 619-4653.

Enhanced BSL- 3 Containment

Situations may arise for which enhancements to BSL-3 practices and equipment are required. An example would be when a BSL-3 laboratory performs diagnostic testing on specimens from patients with hemorrhagic fevers thought to be due to dengue or yellow fever viruses. When the origin of these specimens is Africa, the Middle East, or South America, such specimens might contain etiologic agents, such as arenaviruses, filoviruses, or other viruses that are usually manipulated in a BSL-4 laboratory. Examples of enhancements to BSL-3 laboratories might include 1) enhanced respiratory protection of personnel against aerosols; 2) HEPA filtration of dedicated exhaust air from the laboratory; 3) personal body shower. Additional appropriate training for all animal care personnel should be considered.

VIRUSES WITH BSL-4 CONTAINMENT RECOMMENDATIONS

The recommendations for viruses assigned to BSL-4 containment are based on documented cases of severe and frequently fatal naturally occurring human infections and aerosol-transmitted laboratory infections. SALS recommends that certain agents with a close antigenic relationship to agents assigned to BSL- 4 also be provisionally handled at this level until sufficient laboratory data indicates their retention at this level or movement to work at a lower level.

Laboratory Hazards

The infectious agents may be present in blood, urine, respiratory and throat secretions, semen, and other fluids and tissues from human or animal hosts, and in arthropods, rodents, and NHP. Respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory or animal care personnel.[3,4]

Containment Recommendations

BSL-4 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials of human, animal, or arthropod origin. Clinical specimens from persons suspected of being infected with one of the agents listed in this summary should be submitted to a laboratory with a BSL- 4 maximum containment facility.[5]

DEALING WITH UNKNOWN ARBOVIRUSES

The ACAV has published reports documenting laboratory workers who acquired arbovirus infections during the course of their duties.[6] In the first such document, it was recognized that these laboratory infections typically occurred by unnatural routes such as percutaneous or aerosol exposure, that “lab adapted” strains were still pathogenic for humans, and that as more laboratories worked with newly identified agents, the frequency of laboratory-acquired infections was increasing. Therefore, to assess the risk of these viruses and provide safety guidelines to those working with them, ACAV appointed SALS to evaluate the hazards of working with arboviruses in the laboratory setting.[7,8] The SALS committee made a series of recommendations, published in 1980, describing four levels of laboratory practices and containment guidelines that were progressively more restrictive. These levels were determined after widely-distributed surveys evaluated numerous criteria for each particular virus including 1) past occurrence of laboratory-acquired infections correlated with facilities and practices used; 2) volume of work performed as a measure of potential exposure risk; 3) immune status of laboratory personnel; 4) incidence and severity of naturally-acquired infections in adults; and 5) incidence of disease in animals outside the United States (to assess import risk).

While these criteria are still important factors to consider in any risk assessment for manipulating arboviruses in the laboratory, it is important to note that there have been

many modifications to personal laboratory practices (e.g., working in BSC while wearing extensive personal protective equipment in contrast to working with viruses on an open bench top) and significant changes in laboratory equipment and facilities (e.g. BSC, PAPR) available since the initial SALS evaluation. Clearly, when dealing with a newly recognized arbovirus, there is insufficient previous experience with it; thus, the virus should be assigned a higher biosafety level. However, with increased ability to safely characterize viruses, the relationship to other disease-causing arboviruses can be established with reduced exposure to the investigators. Therefore, in addition to those established by SALS, additional assessment criteria should be considered.

One criterion for a newly identified arbovirus is a thorough description of how the virus will be handled and investigated. For example, experiments involving pure genetic analysis could be handled differently than those where the virus will be put into animals or arthropods.[9] Additionally, an individual risk assessment should consider the fact that not all strains of a particular virus exhibit the same degree of pathogenicity or transmissibility. While variable pathogenicity occurs frequently with naturally identified strains, it is of particular note for strains that are modified in the laboratory. It may be tempting to assign biosafety levels to hybrid or chimeric strains based on the parental types but due to possible altered biohazard potential, assignment to a different biosafety level may be justified.[10] A clear description of the strains involved should accompany any risk assessment.

Most of the identified arboviruses have been assigned biosafety levels; however, a number of those that are infrequently studied, newly identified, or have only single isolation events may not have been evaluated by SALS, ACAV, CDC, or the NIH (See Table 1). Thorough risk assessment is important for all arboviral research and it is of particular importance for work involving unclassified viruses. A careful assessment by the laboratory director, institutional biosafety officer and safety committee, and as necessary, outside experts is necessary to minimize the risk of human, animal, and environmental exposure while allowing research to progress.

Chimeric Viruses

The ability to construct cDNA clones encoding a complete RNA viral genome has led to the generation of recombinant viruses containing a mixture of genes from two or more different viruses. Chimeric, full-length viruses and truncated replicons have been constructed from numerous alphaviruses and flaviviruses. For example, alphavirus replicons encoding foreign genes have been used widely as immunogens against bunyavirus, filovirus, arenavirus, and other antigens. These replicons have been safe and usually immunogenic in rodent hosts leading to their development as candidate human vaccines against several virus groups including retroviruses.[11-14]

Because chimeric viruses contain portions of multiple viruses, the IBC, in conjunction with the biosafety officer and the researchers, must conduct a risk assessment that, in addition to standard criteria, includes specific elements that need to be considered before assigning appropriate biosafety levels and containment practices. These elements

include 1) the ability of the chimeric virus to replicate in cell culture and animal model systems in comparison with its parental strains;[15] 2) altered virulence characteristics or attenuation compared with the parental viruses in animal models;[16] 3) virulence or attenuation patterns by intracranial routes using large doses for agents affecting the CNS;[17,18] and 4) demonstration of lack of reversion to virulence or parental phenotype.

Many patterns of attenuation have been observed with chimeric flaviviruses and alphaviruses using the criteria described above. Additionally, some of these chimeras are in phase II testing as human vaccines.[19]

Chimeric viruses may have some safety features not associated with parental viruses. For example, they are generated from genetically stable cDNA clones without the need for animal or cell culture passage. This minimizes the possibility of mutations that could alter virulence properties. Because some chimeric strains incorporate genomic segments lacking gene regions or genetic elements critical for virulence, there may be limited possibility of laboratory recombination to generate strains exhibiting wild-type virulence.

Ongoing surveillance and laboratory studies suggest that many arboviruses continue to be a risk to human and animal populations. The attenuation of all chimeric strains should be verified using the most rigorous containment requirements of the parental strains. The local IBC should evaluate containment recommendations for each chimeric virus on a case-by-case basis, using virulence data from an appropriate animal model. Additional guidance from the NIH Office of Biotechnology Activities Recombinant DNA Advisory Committee may be necessary.

Agent: West Nile Virus (WNV)

WNV has emerged in recent years in temperate regions of Europe and North America, presenting a threat to public and animal health. This virus belongs to the family *Flaviviridae* and the genus *Flavivirus,* Japanese encephalitis virus antigenic complex. The complex currently includes Alfuy, Cacipacore, Japanese encephalitis, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Rocio, Stratford, Usutu, West Nile, and Yaounde viruses. Flaviviruses share a common size (40-60nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single stranded RNA approximately 10,000-11,000 bases) and virus morphology. The virus was first isolated from a febrile adult woman in the West Nile District of Uganda in 1937.[20] The ecology was characterized in Egypt in the 1950s; equine disease was first noted in Egypt and France in the early 1960s.[21,22] It first appeared in North America in 1999 as encephalitis reported in humans and horses.[23] The virus has been detected in Africa, Europe, the Middle East, west and central Asia, Oceania (subtype Kunjin virus), and most recently, North America.

Occupational Infections

LAI with WNV have been reported in the literature. SALS reported 15 human infections from laboratory accidents in 1980. One of these infections was attributed to aerosol exposure. Two parenteral inoculations have been reported recently during work with animals.[24]

Natural Modes of Infection

In the United States, infected mosquitoes, primarily members of the *Culex* genus, transmit WNV. Virus amplification occurs during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and bird reservoir hosts. People, horses, and most other mammals are not known to develop infectious viremias very often, and thus are probably "dead-end" or incidental hosts.

LABORATORY SAFETY

WNV may be present in blood, serum, tissues, and CSF of infected humans, birds, mammals, and reptiles. The virus has been found in oral fluids and feces of birds. Parenteral inoculation with contaminated materials poses the greatest hazard; contact exposure of broken skin is a possible risk. Sharps precautions should be strictly adhered to when handling potentially infectious materials. Workers performing necropsies on infected animals may be at higher risk of infection.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities with human diagnostic specimens, although it is unusual to recover virus from specimens obtained from clinically ill patients. BSL-2 is recommended for processing field collected mosquito pools whereas BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended, for all manipulations of WNV cultures and for experimental animal and vector studies, respectively.

Dissection of field collected dead birds for histopathology and culture is recommended at BSL-3 containment due to the potentially high levels of virus found in such samples. Non-invasive procedures performed on dead birds (such as oropharyngeal or cloacal swabs) can be conducted at BSL-2.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Agent: Eastern equine encephalitis (EEE) virus, Venezuelan equine encephalitis (VEE) virus, and Western equine encephalitis (WEE) virus

VEE, EEE, and WEE viruses are members of the genus *Alphavirus* in the family *Togaviridae*. They are small, enveloped viruses with a genome consisting of a single strand of positive-sense RNA. All three viruses can cause encephalitis often accompanied by long-term neurological sequelae. Incubation period ranges from 1-10 days and the duration of acute illness is typically days to weeks depending upon severity of illness. Although not the natural route of transmission, the viruses are highly infectious by the aerosol route; laboratory acquired infections have been documented.[25]

Occupational Infections

These alphaviruses, especially VEE virus, are infectious by aerosol in laboratory studies and more than 160 EEE virus, VEE virus, or WEE virus laboratory-acquired infections have been documented. Many infections were due to procedures involving high virus concentrations and aerosol-generating activities such as centrifugation and mouth pipetting. Procedures involving animals (e.g. infection of newly hatched chicks with EEE virus and WEE virus) and mosquitoes also are particularly hazardous.

Natural Modes of Infection

Alphaviruses are zoonoses maintained and amplified in natural transmission cycles involving a variety of mosquito species and either small rodents or birds. Humans and equines are accidental hosts with naturally acquired alphavirus infections resulting from the bites of infected mosquitoes.

EEE virus occurs in focal locations along the eastern seaboard, the Gulf Coast and some inland Midwestern locations of the United States, in Canada, some Caribbean Islands, and Central and South America.[26] Small outbreaks of human disease have occurred in the United States, the Dominican Republic, Cuba, and Jamaica. In the United States, equine epizootics are common occurrences during the summer in coastal regions bordering the Atlantic and Gulf of Mexico, in other eastern and Midwestern states, and as far north as Quebec, Ontario, and Alberta in Canada.

In Central and South America, focal outbreaks due to VEE virus occur periodically with rare large regional epizootics involving thousands of equine cases and deaths in predominantly rural settings. These epizootic/epidemic viruses are theorized to emerge periodically from mutations occurring in the continuously circulating enzootic VEE viruses in northern South America. The classical epizootic varieties of the virus are not present in the U.S. An enzootic subtype, Everglades virus (VEE antigenic complex subtype II virus), exists naturally in southern Florida, while endemic foci of Bijou Bridge virus (VEE antigenic complex subtype III-B virus), have been described in the western United States.[27]

The WEE virus is found mainly in western parts of the United States and Canada. Sporadic infections also occur in Central and South America.

LABORATORY SAFETY

Alphaviruses may be present in blood, CSF, other tissues (e.g., brain), or throat washings. The primary laboratory hazards are parental inoculation, contact of the virus with broken skin or mucus membranes, bites of infected animals or arthropods, or aerosol inhalation.

Containment Recommendations

Diagnostic and research activities involving clinical material, infectious cultures, and infected animals or arthropods should be performed under BSL-3 practices, containment equipment, and facilities. Due to the high risk of aerosol infection, additional personal protective equipment, including respiratory protection, should be considered for non-immune personnel. Animal work with VEE virus, EEE virus and WEE virus should be performed under ABSL-3 conditions. HEPA filtration is required on the exhaust system of laboratory and animal facilities using VEE virus.

SPECIAL ISSUES

Vaccines Two strains of VEE virus (TC-83 and V3526) are highly attenuated in vertebrate studies and have been either exempted (strain TC-83) or excluded (strain V3526) from Select Agent regulations. Because of the low level of pathogenicity, these strains maybe safely handled under BSL-2 conditions without vaccination or additional personal protective equipment.

Investigational vaccine protocols have been developed to immunize at-risk laboratory or field personnel against these alphaviruses, however, the vaccines are available only on a limited basis and may be contraindicated for some personnel. Therefore, additional personal protective equipment may be warranted in lieu of vaccination. For personnel who have no neutralizing antibody titer (either by previous vaccination or natural infection), additional respiratory protection is recommended for all procedures.

Select Agent VEE virus and EEE virus are Select Agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Agent: Rift Valley Fever Virus (RVFV)

RVFV was first isolated in Kenya in 1936 and subsequently shown to be endemically present in almost all areas of sub-Saharan Africa.[28] In periods of heavy rainfall, large epizootics occur involving primarily sheep, cattle, and human disease, although many other species are infected. The primordial vertebrate reservoir is unknown, but the introduction of large herds of highly susceptible domestic breeds in the last few decades has provided a substrate for massive virus amplification. The virus has been introduced into Egypt, Saudi Arabia, and Yemen and caused epizootics and epidemics in those countries. The largest of these was in 1977 to 1979 in Egypt with many thousands of human cases and 610 reported deaths.[29]

Most human infections are symptomatic and the most common syndrome consists of fever, myalgia, malaise, anorexia, and other non-specific symptoms. Recovery within one to two weeks is usual but hemorrhagic fever, encephalitis, or retinitis also occurs. Hemorrhagic fever develops as the primary illness proceeds and is characterized by disseminated intravascular coagulation and hepatitis. Perhaps 2% of cases will develop this complication and the mortality is high. Encephalitis follows an apparent recovery in <1% of cases and results in a substantial mortality and sequelae. Retinal vasculitis occurs in convalescence of a substantial but not precisely known proportion of cases. The retinal lesions are often macular and permanent, leading to substantial loss of visual acuity.

Infected sheep and cattle suffer a mortality rate of 10-35%, and spontaneous abortion occurs virtually in all pregnant females. Other animals studied have lower viremia and lesser mortality but may abort. This virus is an OIE List A disease and triggers export sanctions.

Occupational Infections

The potential for infection of humans by routes other than arthropod transmission was first recognized in veterinarians performing necropsies. Subsequently, it became apparent that contact with infected animal tissues and infectious aerosols were dangerous; many infections were documented in herders, slaughterhouse workers, and veterinarians. Most of these infections resulted from exposure to blood, other tissues including aborted fetal tissues of sick animals.

There have been 47 reported laboratory infections; before modern containment and vaccination became available virtually every laboratory that began work with the virus suffered infections suggestive of aerosol transmission.[30,31]

Natural Modes of Infection

Field studies show RVFV to be transmitted predominantly by mosquitoes, although other arthropods may be infected and transmit. Mechanical transmission also has been documented in the laboratory. Floodwater *Aedes* species are the primary vector and transovarial transmission is an important part of the maintenance cycle.[32] However, many different mosquito species are implicated in horizontal transmission in field studies,

and laboratory studies have shown a large number of mosquito species worldwide to be competent vectors, including North American mosquitoes.

It is currently believed that the virus passes dry seasons in the ova of flood-water *Aedes* mosquitoes. Rain allows infectious mosquitoes to emerge and feed on vertebrates. Several mosquito species can be responsible for horizontal spread, particularly in epizootic/epidemic situations. The vertebrate amplifiers are usually sheep and cattle, with two caveats; as yet undefined native African vertebrate amplifier is thought to exist and very high viremias in humans are thought to play some role in viral amplifications.[33]

Transmission of diseases occurs between infected animals but is of low efficiency and virus titers in throat swabs are low. Nosocomial infection rarely if ever occurs. There are no examples of latency with RVFV, although virus may be isolated from lymphoid organs of mice and sheep for four to six weeks post-infection.

LABORATORY SAFETY

Concentrations of RVFV in blood and tissues of sick animals are often very high. Placenta, amniotic fluid, and fetuses from aborted domestic animals are highly infectious. Large numbers of infectious virus also are generated in cell cultures and laboratory animals.

Containment Recommendations

BSL-3 practices, containment equipment and facilities are recommended for processing human or animal material in endemic zones or in non-endemic areas in emergency circumstances. Particular care should be given to stringent aerosol containment practices, autoclaving waste, decontamination of work areas, and control of egress of material from the laboratory. Other cultures, cells, or similar biological material that could potentially harbor RVFV should not be used in a RVFV laboratory and subsequently removed.

Diagnostic or research studies outside endemic areas should be performed in a BSL-3 laboratory. Personnel also must have additional respiratory protection (such as a PAPR) or be vaccinated for RVFV. In addition, for research conducted in non-endemic areas, the USDA may require full BSL-3Ag containment (See Appendix D).

SPECIAL ISSUES

Vaccines Two apparently effective vaccines have been developed by Department of Defense (DOD) and have been used in volunteers, laboratory staff, and field workers under investigational protocols, but neither vaccine is available at this time.

Select Agent RVFV is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

The live-attenuated MP-12 vaccine strain is specifically exempted from the Select Agent rules. In general, BSL-2 containment is recommended for working with this strain.

The USDA may require enhanced ABSL-3 or ABSL-3 facilities and practices for working with RVFV in the United States (See Appendix D). Investigators should contact the USDA for further guidance before initiating research.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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