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Biosafety in Microbiological and Biomedical Laboratories

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DEDICATION

This fourth edition of *Biosafety in Microbiological and Biomedical Laboratories* is dedicated to the life and achievements of John H. Richardson, D.V.M., M.P.H.

Dr. Richardson was a pioneer in and ceaseless advocate for biological safety and education. He co-edited the first two editions of the BMBL, whose guidelines are now accepted as the international "gold standard" for safely conducting microbiological research. He shaped the programs for quarantining animals imported into the United States and for handling dangerous biological organisms in research laboratories. He was a charter member and former President of the American Biological Safety Association, and helped develop its certification program for biological safety professionals. After a long and distinguished career in the Public Health Service, he served as Director of the Environmental Safety and Health Office of Emory University before becoming a widely sought biosafety consultant.

Perhaps most importantly, Dr. Richardson will be missed by the many friends and associates who were privileged to know and work with him. He was a gentleman and a superb advocate for public health.

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TABLE OF CONTENTS

SECTION I	
<i>Introduction</i>	1
SECTION II	
<i>Principles of Biosafety</i>	8
SECTION III	
<i>Laboratory Biosafety Level Criteria</i>	17
Table 1. Summary of Recommended Biosafety Levels for Infectious Agents.	52
SECTION IV	
<i>Vertebrate Animal Biosafety Level Criteria</i>	53
Table 1. Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals Are Used	75
SECTION V	
<i>Risk Assessment</i>	76
SECTION VI	
<i>Recommended Biosafety Levels for Infectious Agents and Infected Animals</i>	84
SECTION VII	
<i>Agent Summary Statements</i>	88
Section VII-A: Bacterial Agents	88
Section VII-B: Fungal Agents	118
Section VII-C: Parasitic Agents	127
Section VII-D: Prions	134
Section VII-E: Rickettsial Agents	148
Section VII-F: Viral Agents (other than arboviruses)	153
Section VII-G: Arboviruses and Related Zoonotic Viruses	183
Arboviruses Assigned to Biosafety Level 2	183
Table 1. Arboviruses and Arenaviruses Assigned to Biosafety Level 2	186

Table 2. Vaccine Strains of BSL-3/4 Viruses Which May Be Handled at Biosafety Level-2	189
Arboviruses and Arenaviruses Assigned to Biosafety Level 3	189
Table 3. Arboviruses and Certain Other Viruses Assigned to Biosafety Level 3 (on the basis of insufficient experience)	193
Table 4. Arboviruses and Certain Other Viruses Assigned to Biosafety Level 3	194
Arboviruses, Arenaviruses, and Filoviruses Assigned to Biosafety Level 4	196
Table 5. Arboviruses, Arenaviruses and Filoviruses Assigned to Biosafety Level 4	197

APPENDIX A

<i>Primary Containment: Biological Safety Cabinets</i>	200
Table 1. Comparison of Biological Safety Cabinets	205
Figure 2a. Class II, Type A Biological Safety Cabinet	207
Figure 2b. Class II, Type B1 Biological Safety Cabinet	208
Figure 2c. Class II, Type B2 Biological Safety Cabinet	209
Figure 2d. Table-top Model	210
Figure 3. Class III Biological Safety Cabinet	211

APPENDIX B

<i>Immunoprophylaxis</i>	212
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APPENDIX C

<i>Transportation and Transfer of Biological Agents</i>	214
Figure 1. Packing and Labeling of Infectious Substances	219
Figure 2. Packing and Labeling of Clinical Specimens	219

APPENDIX D

<i>Restricted Animal Pathogens</i>	220
------------------------------------	-----

APPENDIX E	
<i>Resources for Information</i>	222
APPENDIX F	
<i>Laboratory Security and Emergency Response for Microbiological and Biomedical Laboratories</i>	224
APPENDIX G	
<i>Integrated Pest Management</i>	230
APPENDIX H	
<i>Working With Human and Other Primate Cells and Tissues</i>	234
APPENDIX I	
<i>Guidelines for Work With Toxins of Biological Origin</i>	237
INDEX	
<i>Index</i>	243

PREFACE

This publication describes the combinations of standard and special microbiological practices, safety equipment, and facilities constituting Biosafety Levels 1-4, which are recommended for work with a variety of infectious agents in various laboratory settings.

These recommendations are advisory. They are intended to provide a voluntary guide or code of practice as well as goals for upgrading operations. They also are offered as a guide and reference in the construction of new laboratory facilities and in the renovation of existing facilities.

However, the application of these recommendations to a particular laboratory operation should be based on a risk assessment of the special agents and activities, rather than used as a universal and generic code applicable to all situations.

Since the publication of the third edition of *Biosafety in Microbiological and Microbiological Laboratories*, a number of events have occurred that influenced some of the changes made in this fourth edition.

- In response to global concern about emerging and re-emerging infectious diseases, the section on Risk Assessment has been enlarged to provide the laboratorian with additional information to make such determinations easier.
- A considerable increase in the design and construction of biomedical and microbiological laboratories has occurred, particularly at Biosafety Levels 3 and 4. In response, clarification of and additions to the "Facilities" sections have been incorporated, particularly in Sections III and IV, as an expansion of our performance-based approach to achieving appropriate containment.
- Interest in prion diseases increased significantly with the identification of bovine spongiform encephalopathy (BSE) in England. In response, an appendix has been added to

address the varied biosafety concerns associated with working with these agents.

- Several laboratory-associated infections have occurred involving both known and previously unknown agents. In response, various Agent Summary Statements have been modified or added to this edition.
- Concern has increased regarding the national and international transfer of infectious microorganisms. Each Agent Summary Statement now contains information regarding the requirements to obtain appropriate permits before transferring the agents from one laboratory to another.
- Finally, growing concerns about bioterrorism have caused considerable interest in biosafety matters in recent years. In response, an additional appendix is designed to help focus attention on the increased security needs of our microbiological laboratories.

We also acknowledge the contributions of many in the science community who have provided ideas for improving this publication. In particular, we are indebted to the Technical Review Committee of the American Biological Safety Association for its thoughtful comments and suggestions.

SECTION I

Introduction

Microbiological laboratories are special, often unique work environments that may pose identifiable infectious disease risks to persons in or near them. Infections have been contracted in the laboratory throughout the history of microbiology. Published reports around the turn of the century describe laboratory-associated cases of typhoid, cholera, glanders, brucellosis, and tetanus.¹ In 1941, Meyer and Eddie² published a survey of 74 laboratory-associated brucellosis infections that had occurred in the United States, and concluded that the "handling of cultures or specimens or the inhalation of dust containing *Brucella* organisms is eminently dangerous to laboratory workers." A number of cases were attributed to carelessness or poor technique in the handling of infectious materials.

In 1949, Sulkin and Pike³ published the first in a series of surveys of laboratory-associated infections. They summarized 222 viral infections, 21 of which were fatal. In at least a third of the cases, the probable source of infection was considered to be associated with the handling of infected animals and tissues. Known accidents were recorded in 27 (12%) of the reported cases.

In 1951, Sulkin and Pike⁴ published the second of the series, based on a questionnaire sent to 5,000 laboratories. Only one-third of the 1,342 cases cited had been reported in the literature. Brucellosis outnumbered all other reported laboratory-acquired infections and, together with tuberculosis, tularemia, typhoid, and streptococcal infection, accounted for 72% of all bacterial infections and for 31% of infections caused by all agents. The overall case fatality rate was 3%. Only 16% of all infections reported were associated with a documented accident. The majority of these were related to mouth pipetting and the use of needle and syringe.

This survey was updated in 1965,⁵ adding 641 new or previously unreported cases, and again in 1976,⁶ summarizing a cumulative total of 3,921 cases. Brucellosis, typhoid, tularemia,

Introduction

tuberculosis, hepatitis, and Venezuelan equine encephalitis were the most commonly reported infections. Fewer than 20% of all cases were associated with a known accident. Exposure to infectious aerosols was considered to be a plausible but unconfirmed source of infection for the more than 80% of the reported cases in which the infected person had "worked with the agent."

In 1967, Hanson et al⁷ reported 428 overt laboratory-associated infections with arboviruses. In some instances, the ability of a given arbovirus to produce human disease was first confirmed as the result of unintentional infection of laboratory personnel. Exposure to infectious aerosols was considered the most common source of infection.

In 1974, Skinhoj⁸ published the results of a survey which showed that personnel in Danish clinical chemistry laboratories had a reported incidence of hepatitis (2.3 cases per year per 1,000 employees) seven times higher than that of the general population. Similarly, a 1976 survey by Harrington and Shannon⁹ indicated that medical laboratory workers in England had "a five times increased risk of acquiring tuberculosis compared with the general population." Hepatitis B and shigellosis were also shown to be continuing occupational risks. Along with tuberculosis, these were the three most commonly reported occupation-associated infections in Britain.

Although these reports suggest that laboratory personnel were at increased risk of being infected by the agents they handle, actual rates of infection are typically not available. However, the studies of Harrington and Shannon⁹ and of Skinhoj¹⁰ indicate that laboratory personnel had higher rates of tuberculosis, shigellosis, and hepatitis B than does the general population.

In contrast to the documented occurrence of laboratory--acquired infections in laboratory personnel, laboratories working with infectious agents have not been shown to represent a threat to the community. For example, although 109 laboratory-associated infections were recorded at the Centers for Disease Control and Prevention from 1947-1973,¹¹ no secondary

Introduction

cases were reported in family members or community contacts. The National Animal Disease Center reported a similar experience,¹² with no secondary cases occurring in laboratory and non-laboratory contacts of 18 laboratory-associated cases occurring from 1960-1975. A secondary case of Marburg disease in the wife of a primary case was presumed to have been transmitted sexually two months after his dismissal from the hospital.¹³ Three secondary cases of smallpox were reported in two laboratory associated outbreaks in England in 1973¹⁴ and 1978.¹⁵ There were earlier reports of six cases of Q fever among personnel of a commercial laundry that cleaned linens and uniforms from a laboratory working with the agent,¹⁶ one case of Q fever in a visitor to a laboratory,¹⁷ and two cases of Q fever in household contacts of a rickettsiologist.¹⁸ One case of Monkey B virus transmission from an infected animal care giver to his wife has been reported, apparently due to contact of the virus with broken skin.¹⁹ These cases are representative of the sporadic nature and infrequency of community infections in laboratory personnel working with infectious agents.

In his 1979 review,²⁰ Pike concluded that "the knowledge, the techniques, and the equipment to prevent most laboratory infections are available." In the United States, however, no single code of practice, standards, guidelines, or other publication provided detailed descriptions of techniques, equipment, and other considerations or recommendations for the broad scope of laboratory activities conducted with a variety of indigenous and exotic infectious agents. The booklet, *Classification of Etiologic Agents on the Basis of Hazard*,²¹ served as a general reference for some laboratory activities utilizing infectious agents. This booklet, and the concept of categorizing infectious agents and laboratory activities into four classes or levels, served as a basic format for earlier editions of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). This fourth edition of the BMBL continues to specifically describe combinations of microbiological practices, laboratory facilities, and safety equipment, and to recommend their use in four categories or biosafety levels of laboratory operation with selected agents infectious to humans.

Introduction

The descriptions of Biosafety Levels 1-4 parallel those in the *NIH Guidelines for Research Involving Recombinant DNA*,^{22,23} and are consistent with the general criteria originally used in assigning agents to Classes 1-4 in *Classification of Etiologic Agents on the Basis of Hazard*.²⁴ Four biosafety levels are also described for infectious disease activities utilizing small laboratory animals. Recommendations for biosafety levels for specific agents are made on the basis of the potential hazard of the agent and of the laboratory's function or activity.

Since the early 1980s, laboratories have applied these fundamental guidelines in activities associated with manipulations involving the human immunodeficiency virus (HIV). Even before HIV was identified as the causative agent of Acquired Immunodeficiency Syndrome (AIDS), the principles for manipulating a bloodborne pathogen were suitable for safe laboratory work. Guidelines were also promulgated for health care workers under the rubric of Universal Precautions.²⁵ Indeed, Universal Precautions and this publication have become the basis for the safe handling of blood and body fluids, as described in the recent OSHA publication, *Bloodborne Pathogen Standard*.²⁶

In the late 1980s, considerable public concern was expressed about medical wastes, which led to the promulgation of the Medical Waste Tracking Act of 1988.²⁷ The principles established in the earlier volumes of the BMBL for handling potentially infectious wastes as an occupational hazard were reinforced by the National Research Council's *Biosafety in the Laboratory: Prudent Practices for the Handling and Disposal of Infectious Materials*.²⁸

As this edition goes to press, there is growing concern about the re-emergence of *M. tuberculosis* and worker safety in laboratory and health care settings. The BMBL's underlying principles, which seek to ensure safe practices, procedures and facilities, are applicable to the control of this airborne pathogen, including its multi-drug-resistant strains.^{29,30} In addition, recombinant DNA technologies are being applied routinely in the laboratory to modify the genetic composition of various microorganisms. A thorough risk assessment must be

Introduction

conducted when addressing these activities and their inherent unknowns.

Experience has demonstrated the prudence of the Biosafety Level 1-4 practices, procedures, and facilities described for manipulations of etiologic agents in laboratory settings and animal facilities. Although no national reporting system exists for reporting laboratory-associated infections, anecdotal information suggests that strict adherence to these guidelines does contribute to a healthier and safer work environment for laboratorians, their co-workers, and the surrounding community. To further reduce the potential for laboratory-associated infections, the guidelines presented here should be considered minimal guidance for containment. They must be customized for each individual laboratory and can be used in conjunction with other available scientific information.

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SECTION II

Principles of Biosafety

The term "containment" is used in describing safe methods 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.

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by both good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection. *Secondary containment*, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. Therefore, the three elements of containment include laboratory practice and technique, safety equipment, and facility design. 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 to handle 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

Principles of Biosafety

associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or material. 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). Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II biological safety cabinets are primary barriers which 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 this hazard, containment controls such as BSCs or centrifuge cups must be

Principles of Biosafety

used when handling infectious agents that can be transmitted through the aerosol route of exposure.

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 biological safety cabinets and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in biological safety cabinets, 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 which may be accidentally released from the laboratory. Laboratory management is 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 Biosafety Level 1 and 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 handwashing 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

Principles of Biosafety

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 *Applications Handbook for Heating, Ventilation, and Air-Conditioning (HVAC)* published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).¹

Biosafety Levels. Four biosafety levels (BSLs) are described in Section III, 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 recommended biosafety level(s) for the organisms in Section VII (Agent Summary Statements) represent those conditions under which the agent ordinarily can be safely handled. The laboratory director is specifically and primarily responsible for assessing the risks and appropriately applying the recommended biosafety levels. Generally, work with known agents should be conducted at the biosafety level recommended in Section VII. When specific 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.

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 Recombinant DNA Guidelines are representa-

Principles of Biosafety

tive 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.

Biosafety Level 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. Biosafety Level 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 Biosafety Level 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

Principles of Biosafety

devices such as a BSC or safety centrifuge cups. Other primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers such as handwashing 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 Biosafety Level 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 Biosafety Level 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

Principles of Biosafety

lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4.

The primary hazards to personnel working with Biosafety Level 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 Biosafety Level 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, and the nature or function of the laboratory may further influence the director in applying these recommendations.

Animal Facilities. Four biosafety levels are also described for activities involving infectious disease work with 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.

Principles of Biosafety

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 which 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 Biosafety Level 2, the recommended level for work with bloodborne pathogens such as hepatitis B virus and HIV. The containment elements described in Biosafety Level 2 are consistent with the OSHA standard, "*Occupational Exposure to Bloodborne Pathogens*"^{3,4}, from the Occupational Safety and Health Administration. This requires the use of specific precautions with **all** clinical specimens of blood or other potentially infectious material (Universal or Standard Precautions).⁵ Additionally, other recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.⁶

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

Principles of Biosafety

tory 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 U. S. Department of Agriculture 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 D).

References:

1. American Society of Heating, Refrigerating, and Air-Conditioning Engineers, Inc. 1999. "Laboratories." In: ASHRAE Handbook, *Heating, Ventilation, and Air-Conditioning Applications*, Chapter 13.
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4. Richmond, J.Y. 1994. "HIV Biosafety: Guidelines and Regulations." In (G. Schochetman, J. R. George, Eds.), *AIDS Testing*, Edition 2 (pp. 346-360). Springer-Verlag New York, Inc.
5. Centers for Disease Control. 1988. Update: Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus and Other Bloodborne Pathogens in Healthcare Settings. *MMWR*, **37**:377-382, 387, 388.
6. National Committee for Clinical Laboratory Standards (NCCLS). 1997. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue. Approved guideline. Dec. 1977, NCCLS Doc. M29-A (ISBN1-56238-339-6).

SECTION III

Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Tables 1 of this section and Section IV (see pages 52 and 75). The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1 (BSL-1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear con-

Laboratory Biosafety Level Criteria – Biosafety Level 1

tact lenses in laboratories should also wear goggles or a face shield . Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
9. A biohazard sign may be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.
10. An insect and rodent control program is in effect (see Appendix G).

B. *Special Practices* None

Laboratory Biosafety Level Criteria – Biosafety Level 1

C. Safety Equipment (Primary Barriers)

Laboratory Biosafety Level Criteria – Biosafety Level 2

1. Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.
4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

D. *Laboratory Facilities (Secondary Barriers)*

1. Laboratories should have doors for access control.
2. Each laboratory contains a sink for handwashing.
3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
6. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

Biosafety Level 2 (BSL-2)

Laboratory Biosafety Level Criteria – Biosafety Level 2

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash

Laboratory Biosafety Level Criteria – Biosafety Level 2

- of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
 9. An insect and rodent control program is in effect (see Appendix G).

B. *Special Practices*

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal

Laboratory Biosafety Level Criteria – Biosafety Level 2

protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

Laboratory Biosafety Level Criteria – Biosafety Level 2

- b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - c. Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.
 - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.
9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

Laboratory Biosafety Level Criteria – Biosafety Level 2

11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
12. Animals not involved in the work being performed are not permitted in the lab.

C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory.

Laboratory Biosafety Level Criteria – Biosafety Level 2

This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. *Laboratory Facilities* (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for handwashing.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and

Laboratory Biosafety Level Criteria – Biosafety Level 3

other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
8. An eyewash station is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. There are no specific ventilation requirements. 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. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

Biosafety Level 3 (BSL-3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropri-

Laboratory Biosafety Level Criteria – Biosafety Level 3

ate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that some existing facilities may not have all the facility features recommended for Biosafety Level 3 (i.e., double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.), may be achieved in a Biosafety Level 2 facility, **providing** 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment and facilities apply to agents assigned to Biosafety Level 3:

A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

Laboratory Biosafety Level Criteria – Biosafety Level 3

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.
9. An insect and rodent control program is in effect (see Appendix G).

B. *Special Practices*

1. Laboratory doors are kept closed when experiments are in progress.
2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may have serious consequences are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.

Laboratory Biosafety Level Criteria – Biosafety Level 3

3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.
5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.
6. Baseline serum samples are collected as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.
7. A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
8. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent expo-

Laboratory Biosafety Level Criteria – Biosafety Level 3

tures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.

9. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.
10. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
 - b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

Laboratory Biosafety Level Criteria – Biosafety Level 3

- c. Syringes which re-sheathe the needle, needleless systems, and other safe devices are used when appropriate.
 - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.
11. All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean-up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.
12. Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.
- a. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.
 - b. Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.
13. Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during

Laboratory Biosafety Level Criteria – Biosafety Level 3

collection, handling, processing, storage, transport, or shipping.

14. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.
15. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
16. Animals and plants not related to the work being conducted are not permitted in the laboratory.

C. *Safety Equipment* (Primary Barriers)

1. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.
2. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
3. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
4. All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs, etc., are conducted in a Class II or Class III biological safety cabinet (see Appendix A).
5. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate

Laboratory Biosafety Level Criteria – Biosafety Level 3

combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.

6. Respiratory and face protection are used when in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable (see Appendix F). A clothes change room may be included in the passageway.
2. Each laboratory room contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the room exit door.
3. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of coved floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed. Openings such as around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.

Laboratory Biosafety Level Criteria – Biosafety Level 3

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
6. All windows in the laboratory are closed and sealed.
7. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.
8. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.
9. A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms

Laboratory Biosafety Level Criteria – Biosafety Level 3

should be considered to notify personnel of HVAC system failure.

10. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets (see Appendix A).
11. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
12. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).
13. An eyewash station is readily available inside the laboratory.
14. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
15. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be

Laboratory Biosafety Level Criteria – Biosafety Level 4

tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

Biosafety Level 4 (BSL-4)

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents are handled at this level until sufficient data are obtained either to confirm continued work at this level, or to work with them at a lower level. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They are supervised by competent scientists who are trained and experienced in working with these agents. Access to the laboratory is strictly controlled by the laboratory director. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted.

Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

Laboratory Biosafety Level Criteria – Biosafety Level 4

The following standard and special safety practices equipment, and facilities apply to agents assigned to Biosafety Level 4:

A. Standard Microbiological Practices

1. Access to the laboratory is limited by the laboratory director when experiments are in progress.
2. Policies for safe handling of sharps are instituted.
3. All procedures are performed carefully to minimize the creation of aerosols.
4. Work surfaces are decontaminated at least once a day and after any spill of viable material.
5. All waste is decontaminated before disposal by an approved method such as autoclaving.
6. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices

1. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Therefore, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as children or pregnant women, are not allowed in the laboratory or animal rooms.

The *supervisor* has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazard control

Laboratory Biosafety Level Criteria – Biosafety Level 4

officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook, signed by all personnel, indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

2. When infectious materials or infected animals are present in the laboratory or animal rooms, hazard warning signs, incorporating the universal biohazard symbol, are posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).
3. The *laboratory director* is responsible for ensuring that, before working with organisms at Biosafety Level 4, all personnel demonstrate a high proficiency in standard microbiological practices and techniques, and in the special practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in these unique safe microbiological practices and techniques.
4. Laboratory personnel receive available immunizations for the agents handled or potentially present in the laboratory.
5. Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for

Laboratory Biosafety Level Criteria – Biosafety Level 4

the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.

6. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.
8. Personnel enter and leave the laboratory only through the clothing change and shower rooms. They take a decontaminating shower each time they leave the laboratory. Personnel use the airlocks to enter or leave the laboratory only in an emergency.
9. Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the laboratory. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing in the inner change room. Soiled clothing is autoclaved before laundering.
10. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

Laboratory Biosafety Level Criteria – Biosafety Level 4

11. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
 - b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - c. Syringes that re-sheath the needle, needleless systems, and other safety devices are used when appropriate.
 - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass must be decontaminated before disposal, according to any local, state, or federal regulations.
12. Biological materials to be removed from the Class III cabinet or from the Biosafety Level 4 laboratory in a viable or intact state are transferred to a nonbreakable, sealed primary container and then enclosed in a non-

Laboratory Biosafety Level Criteria – Biosafety Level 4

breakable, sealed secondary container. This is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

13. No materials, except biological materials that are to remain in a viable or intact state, are removed from the Biosafety Level 4 laboratory unless they have been autoclaved or decontaminated before they leave the laboratory. Equipment or material that might be damaged by high temperatures or steam may be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.
14. Laboratory equipment is decontaminated routinely after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Equipment is decontaminated before it is sent for repair or maintenance.
15. Spills of infectious materials are contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with concentrated infectious material. A spill procedure is developed and posted within the laboratory.
16. A system is established for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for the quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.
17. Materials not related to the experiment being conducted (e.g., plants, animals, and clothing) are not permitted in the facility.

Laboratory Biosafety Level Criteria – Biosafety Level 4

C. Safety Equipment (Primary Barriers)

All procedures within the facility are conducted in the Class III biological safety cabinet or in Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system.

D. Laboratory Facility (Secondary Barriers)

There are two models for Biosafety Level 4 laboratories: (A) the Cabinet Laboratory where all handling of the agent is performed in a Class III Biological Safety Cabinet, and (B) the Suit Laboratory where personnel wear a protective suit. Biosafety Level-4 laboratories may be based on either model or a combination of both models in the same facility. If a combination is used, each type must meet all the requirements identified for that type.

(A) Cabinet Laboratory (See Appendix A)

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through a minimum of two doors prior to entering the room(s) containing the Class III biological safety cabinet (cabinet room). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the cabinet room. A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom for decontamination is provided at the containment barrier for passage of those materials, supplies, or equipment that are not brought into the cabinet room through the change room.
2. Daily inspections of all containment parameters (e.g., directional airflow) and life support systems are completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

Laboratory Biosafety Level Criteria – Biosafety Level 4

3. Walls, floors, and ceilings of the cabinet room and inner change room are constructed to form a sealed internal shell which facilitates fumigation and is resistant to entry and exit of animals and insects. Floors are integrally sealed and coved. The internal surfaces of this shell are resistant to liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Openings around doors into the cabinet room and inner change room are minimized and are capable of being sealed to facilitate decontamination. Any drains in the cabinet room floor are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters and protection against vermin.
4. Bench tops have seamless or sealed surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
5. Laboratory furniture is of simple open construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
6. A hands-free or automatically operated handwashing sink is provided near the door of the cabinet room(s) and the outer and inner change rooms.
7. If there is a central vacuum system, it does not serve areas outside the cabinet room. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other

Laboratory Biosafety Level Criteria – Biosafety Level 4

liquid and gas services to the cabinet room are protected by devices that prevent backflow.

8. If water fountains are provided, they are automatically or foot-operated and are located in the facility corridors outside the laboratory. The water service to the fountain is isolated from the distribution system supplying water to the laboratory areas and is equipped with a backflow preventer.
9. Access doors to the laboratory are self-closing and lockable.
10. Any windows are breakage-resistant and sealed.
11. Double-door autoclaves are provided for decontaminating materials passing out of both the Class III biological safety cabinet(s) and the cabinet room(s). Autoclaves that open outside of the containment barrier must be sealed to the wall of the containment barrier. The autoclave doors are automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.
12. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the Class III biological safety cabinet(s) and the cabinet room(s).
13. Liquid effluents from the dirty-side inner change room (including toilets) and cabinet room sinks, floor drains (if used), autoclave chambers, and other sources within the cabinet room are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and clean-side toilets may be discharged to the sanitary sewer without treatment.

Laboratory Biosafety Level Criteria – Biosafety Level 4

The process used for decontamination of liquid wastes must be validated physically and biologically.

14. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate any system malfunction. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the cabinet room is provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and the HVAC control system is designed to prevent sustained positive pressurization of the laboratory. The Class III cabinet should be directly connected to the exhaust system. If the Class III cabinet is connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinet.
15. The supply air to and exhaust air from the cabinet room, inner change room, and anteroom pass through HEPA filter(s). The air is discharged away from occupied spaces and air intakes. The HEPA filter(s) are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for *in situ* decontamination of the filter prior to removal, or removal of the filter in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust

Laboratory Biosafety Level Criteria – Biosafety Level 4

HEPA filters can be extended through adequate prefiltration of the supply air.

16. The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.
17. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, computer).

(B) *Suit Laboratory*

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through the changing and decontamination areas prior to entering the room(s) where work is done with BSL-4 agents (suit area). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the suit area. A specially designed suit area is maintained in the facility to provide personnel protection equivalent to that provided by Class III biological safety cabinets. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system protected by HEPA filtration. The life support system includes redundant breathing air compressors, alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. An automatically starting emergency power source is provided at a minimum for the exhaust system, life support

Laboratory Biosafety Level Criteria – Biosafety Level 4

systems, alarms, lighting, entry and exit controls, and BSCs.. The air pressure within the suit is positive to the surrounding laboratory. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area, chemical shower, and airlocks, are sealed.

2. A daily inspection of all containment parameters (e.g., directional airflow, chemical showers) and life support systems is completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.
3. A double-doored autoclave is provided at the containment barrier for decontaminating waste materials to be removed from the suit area. The autoclave door, which opens to the area external to the suit area, is sealed to the outer wall of the suit area and is automatically controlled so that the outside door can be opened only after the autoclave "sterilization" cycle. A dunk tank, fumigation chamber, or ventilated airlock for decontamination is provided for passage of materials, supplies, or equipment that are not brought into the suit area through the change room. These devices can be also used for the safe removal of materials, supplies, or equipment from the laboratory that cannot be decontaminated in the autoclave.
4. Walls, floors, and ceilings of the suit area are constructed to form a sealed internal shell, which facilitates fumigation and is animal and insect prohibitive (see Appendix G). The internal surfaces of this shell are resistant to liquids and chemicals, facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floor of the suit area contain traps filled with a chemical disinfectant of

Laboratory Biosafety Level Criteria – Biosafety Level 4

demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters.

5. Internal facility appurtenances in the suit area, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area.
6. Bench tops have seamless surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
7. Laboratory furniture is of simple open construction capable of supporting anticipated loading and uses. Non-porous materials are preferable. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
8. A hands-free or automatically operated handwashing sink is provided in the suit area(s); handwashing sinks in the outer and inner change rooms should be considered based on the risk assessment.
9. If there is a central vacuum system, it does not serve areas outside the suit area. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the suit area are protected by devices that prevent backflow.
10. Access doors to the laboratory are self-closing and lockable. Inner and outer doors to the chemical shower and inner and outer doors to airlocks are

Laboratory Biosafety Level Criteria – Biosafety Level 4

interlocked to prevent both doors from being opened simultaneously.

11. Any windows are breakage-resistant and are sealed.
12. Liquid effluents from sinks, floor drains (if used), autoclave chambers and other sources within the containment barrier are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.
13. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. Redundant supply fans are recommended. Redundant exhaust fans are required. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate malfunction of the system. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the suit area must be provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and an HVAC control system is installed to prevent positive pressurization of the laboratory.
14. The supply air to the suit area, decontamination shower, and decontamination airlock is protected by passage through a HEPA filter. The general room exhaust air from the suit area, decontamination shower and decontamination airlock is treated by a passage through two HEPA filters in series prior to discharge to the outside. The air is discharged away from occupied spaces and air intakes. The HEPA

Laboratory Biosafety Level Criteria – Biosafety Level 4

filters are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for *in situ* decontamination of the filter prior to removal. Alternatively, the filter can be removed in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust HEPA filters can be extended through adequate prefiltration of the supply air.

15. The positioning of the supply and exhaust points should be such that dead air space in the suit room is minimized.
16. The treated exhaust air from Class II biological safety cabinets, located in a facility where workers wear a positive pressure suit, may be discharged into the room environment or to the outside through the facility air exhaust system. If the treated exhaust is discharged to the outside through the facility exhaust system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust system.
17. The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.
18. Appropriate communication systems should be provided between the laboratory and the outside.

Table 1. Summary of Recommended Biosafety Levels for Infectious Agents.

Biosafety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: <ul style="list-style-type: none"> ● 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: <ul style="list-style-type: none"> ● Controlled access ● Decontamination of all waste ● Decontamination of lab clothing before laundering ● Baseline serum 	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: <ul style="list-style-type: none"> ● Physical separation from access corridors ● Self-closing, double-door access ● Exhausted air not recirculated ● Negative airflow into laboratory

4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: <ul style="list-style-type: none"> ● 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 <u>in combination with</u> full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: <ul style="list-style-type: none"> ● Separate building or isolated zone ● Dedicated supply and exhaust, vacuum, and decon systems ● Other requirements outlined in the text
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SECTION IV

Vertebrate Animal Biosafety Level Criteria

If experimental animals are used, institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, and care. Laboratory animal facilities are simply 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.

However, it is well to remember that the animal room can present some unique problems. In the microbiological laboratory, hazardous conditions are caused by personnel or by the equipment being used. In the animal room, the activities of the animals themselves can present new hazards. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic disease.

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 should have an occupational health and safety plan. The recent publication of the Institute of Medicine, *Occupational Health and Safety in the Care of Research Animals*,³ is most helpful in this regard.

Ideally, facilities for laboratory animals used in studies of infectious or noninfectious 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 considered in the plans. A "clean/dirty hall" layout may be useful to minimize this risk.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 1

experiments with animals infected with agents that cause, or may cause, human infection. 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 inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in the standards for commonly used laboratory animals. *Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates*,⁴ prepared by the Subcommittee on Arbovirus Laboratory Safety (SALS) of the American Committee on Arthropod-Borne Viruses, serves as a useful reference in the design and operation of facilities using arthropods.

Animal Biosafety Level 1 (ABSL-1)

Animal Biosafety Level 1 (ABSL-1) is suitable for work involving well characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.

A. Standard Practices

1. The animal facility director establishes policies, procedures, and protocols for emergency situations. Each project is subject to pre-approval by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biohazard Committee (IBC). Any special practices are approved at this time.
2. Only those persons required for program or support purposes are authorized to enter the facility. Before

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 1

- entering, persons are advised of the potential biohazards and are instructed on the appropriate safeguards.
3. An appropriate medical surveillance program is in place.
 4. A safety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.
 5. 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.
 6. All procedures are carefully performed to minimize the creation of aerosols or splatters.
 7. Work surfaces are decontaminated after use or after any spill of viable materials.
 8. All wastes from the animal room (including animal tissues, carcasses, and contaminated bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended.
 9. Policies for the safe handling of sharps are instituted.
 10. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
 11. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators).

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 1

12. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices: None.

C. Safety Equipment (Primary Barriers):

1. The wearing of laboratory coats, gowns, and/or uniforms in the facility is recommended. Laboratory coats remain in the animal room. Gowns and uniforms are not worn outside the facility.
2. Persons having contact with non-human primates should assess their risk of mucous membrane exposure and wear appropriate eye and face protection.⁵

D. Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
2. External facility doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
4. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
5. Windows are not recommended. Any 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.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

6. If floor drains are provided, the traps are always filled with water and/or an appropriate disinfectant.
7. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*, latest edition.⁶ No recirculation of exhaust air should occur. It is recommended that animal rooms maintain negative pressure compared to adjoining hallways.
8. The facility has a hand washing sink.
9. Cages are washed manually or in a 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.

Animal Biosafety Level 2 (ABSL-2)

Animal Biosafety Level 2 involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.

A. Standard Practices

1. Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biohazard Committee (IBC).
2. Access to the animal room is limited to the fewest number of individuals possible. Personnel who must enter the

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

room for program or service purposes when work is in progress are advised of the potential hazard.

3. An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or potentially present (e.g., hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented.⁷
4. A biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.
5. 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.
6. All procedures are carefully performed to minimize the creation of aerosols or splatters.
7. Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
8. All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. The outer surface of the containers is disinfected prior to moving the material. Autoclaving of the contents prior to incineration is recommended.
9. Policies for the safe handling of sharps are instituted:

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

- a. Needles and syringes or other sharp instruments are restricted for use in the animal facility only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Syringes that re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
 - c. Plasticware should be substituted for glassware whenever possible.
10. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
 11. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements (e.g., the need for immunizations and respirators) for entering the animal room.
 12. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices

1. Animal care laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. Records of all training provided are maintained. In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal facility unless special procedures can eliminate the extra risk.

Vertebrate Animal Biosafety Level Criteria – Animal Biosafety Level 2

2. Only animals used for the experiment(s) are allowed in the room.
3. All equipment must be appropriately decontaminated prior to removal from the room.
4. Spills and accidents which result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

C. Safety Equipment (Primary Barriers)

1. Gowns, uniforms, or laboratory coats are worn while in the animal room. The laboratory coat is removed and left in the animal room. Gowns, uniforms, and laboratory coats are removed before leaving the animal facility. Gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.
2. Personal protective equipment is used based on risk assessment determinations (see Section V). Appropriate face/eye and respiratory protection is worn by all personnel entering animal rooms that house nonhuman primates.⁸
3. Biological safety cabinets, other physical containment devices, and/or personal protective equipment (e.g., respirators, face shields) are used whenever conducting procedures with a high potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals.
4. When needed, animals are housed in primary biosafety containment equipment appropriate for the animal species. Filter top cages are always handled in properly

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 2

designed and operating animal biocontainment cabinets recommended for rodents.

D. Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
2. Access to the facility is limited by secure locked doors. External doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
4. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
5. Windows are not recommended. Any windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are to be fitted with fly screens.
6. If floor drains are provided, the traps are always filled with an appropriate disinfectant.
7. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation should be provided in accordance with criteria from *Guide for Care and Use of Laboratory Animals*, latest edition. The direction of airflow in the animal facility is inward; animal rooms should maintain negative pressure compared to adjoining hallways.

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 3

8. Cages are washed manually or in an appropriate cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
9. An autoclave is available in the animal facility to decontaminate infectious waste.
10. A hand washing sink is in the animal room where infected animals are housed, as well as elsewhere in the facility.
11. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

Animal Biosafety Level 3 (ABSL-3)

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

A. Standard Practices

1. Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
2. The laboratory or animal facility director limits access to the animal room to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
3. An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 3

for the agents handled or potentially present (e.g., hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented.⁹ In general, persons who may be at increased risk of acquiring infection, or for whom infection might have serious consequences, are not allowed in the animal facility unless special procedures can eliminate the extra risk. Assessment should be made by the occupational health physician.

4. A biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.
5. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should be done only in designated areas and are not permitted in animal or procedure rooms.
6. All procedures are carefully performed to minimize the creation of aerosols or splatters.
7. Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
8. All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse animal tissues) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended. The outer surface of the containers is disinfected prior to moving the material (see Special Practices #3 below).
9. Policies for the safe handling of sharps are instituted.
 - a. Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 3

- is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
- b. Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
 - c. Plasticware should be substituted for glassware whenever possible.
10. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
 11. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators).
 12. All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s).
 13. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. As necessary, personnel receive updates and/or additional training on procedural or policy changes. Records of all training provided are maintained.
 14. An insect and rodent control program is in effect.

B. Special Practices

1. Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. Equipment must be decontaminated

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 3

according to any local, state, or federal regulations before being packaged for transport or removal from the facility for repair or maintenance.

2. A spill procedure is developed and posted. Only personnel properly trained and equipped to work with infectious materials are to clean up spills. Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
3. All wastes from the animal room must be autoclaved prior to incineration or other appropriate terminal treatment.
4. Materials not related to the experiment (e.g., plants, animals) are not permitted in the animal room.

C. Safety Equipment (Primary Barriers)

1. Uniforms or scrub suits are worn by personnel entering the animal room. Wrap-around or solid-front gowns should be worn over this clothing. Front-button laboratory coats are unsuitable. The gown must be removed and left in the animal room. Before leaving the animal facility, scrub suits and uniforms are removed and appropriately contained and decontaminated prior to laundering or disposal.
2. Personal protective equipment used is based on risk assessment determinations.
 - a. Personal protective equipment is used for all activities involving manipulations of infectious material or infected animals.
 - b. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before disposal.

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 3

- c. Appropriate face/eye and respiratory protection (e.g., respirators and face shields) is worn by all personnel entering animal rooms.
 - d. Boots, shoe covers, or other protective footwear, and disinfectant foot baths are available and used where indicated.
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 open cages placed in inward flow ventilated enclosures (e.g., laminar flow cabinets), solid wall and bottom cages covered with filter bonnets, or other equivalent primary containment systems.
 4. Biological safety cabinets and other physical containment devices are used whenever conducting procedures with a potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals. At BSL-3, all work should be done in a primary barrier; otherwise respirators should be worn by personnel in the room.

D. Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
2. Access to the facility is limited by a self-closing and self-locking door. This exterior entry door may be controlled by a key lock, card key, or proximity reader. Entry into the animal room is via a double-door entry which may include a change room and shower(s). An additional double-door access (air-lock) or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility, respectively. Doors to animal rooms open inward and are self-closing. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 3

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 and openings around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.
4. A hands-free or automatically operated hand washing sink is provided in each animal room near the exit door. The sink trap is filled with an appropriate disinfectant after each use.
5. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
6. Windows are not recommended. Any windows must be resistant to breakage and must be sealed.
7. If floor drains are provided, they are always filled with an appropriate disinfectant.
8. Ventilation should be provided in accordance with criteria from the *Guide for Care and Use of Laboratory Animals*, latest edition. A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from “clean” areas and toward “contaminated” areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, and 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

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 3

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 HVAC system failure.

9. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the animal room if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used, they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets (see Appendix A).
10. Cages are washed in a cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F.
11. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious waste before moving it to other areas of the facility.
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. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 4

14. The completed Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified at least annually against these procedures as modified by operational experience.
15. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state, or local regulations.

Animal Biosafety Level 4 (ABSL-4)

Animal Biosafety Level 4 involves practices suitable for addressing dangerous or exotic agents that pose high risk of life threatening disease, aerosol transmission, or related agents with unknown risk of transmission. ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. Procedures must be developed locally to address specific operations of the Class III cabinet line or the suit laboratory.

A. Standard Practices

1. Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
2. The laboratory or animal facility director limits access to the animal room to the fewest individuals possible. Personnel who must enter the room for program or

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 4

service purposes when work is in progress are advised of the potential hazard.

3. A medical surveillance program must be instituted for all persons entering an ABSL-4 facility. This program must include appropriate immunizations, serum collection, and availability of post-exposure counseling and potential prophylaxis.¹⁰ In general, persons who may be at increased risk of acquiring infection, or for whom infection might have serious consequences, are not allowed in the animal facility unless special procedures can eliminate the extra risk. Assessment should be made by the occupational health physician.
4. A site-specific biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures.
5. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should be done only in designated areas and are not permitted in animal or procedure rooms.
6. All procedures are carefully performed to minimize the creation of aerosols or splatters.
7. Equipment and work surfaces in the room are routinely decontaminated with an appropriate disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
8. A spill procedure is developed and posted. Only personnel properly trained and equipped to work with infectious materials are to clean up spills. Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 4

- are provided as appropriate and written records are maintained.
9. All wastes (including animal tissues, carcasses, and contaminated bedding), other materials for disposal, and clothing to be laundered, are sterilized in a double-door autoclave located in the secondary barrier wall of the facility (see B-4 below). Disposable wastes are incinerated.
 10. Policies for the safe handling of sharps are instituted.
 - a. Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Syringes that re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
 - c. Plasticware should be substituted for glassware whenever possible.
 11. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators).
 12. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. Records are maintained on all training provided.
 13. Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned.

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 4

and washed. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with infectious materials, and especially after spills, splashes, or other contamination by infectious materials. Equipment must be decontaminated according to any local, state, or federal regulations before removal from the facility for repair or maintenance.

14. Personnel assigned to work with infected animals should work in pairs. Based on the risk assessment (see Section V), use of squeeze cages, working only with anesthetized animals, or other appropriate procedures to reduce possible worker exposure must be instituted.
15. Materials not related to the experiment (e.g., plants, animals) are not permitted in the facility.

B. Special Practices

1. Additional measures are effected to control access (e.g., 24-hour guard and check in/out system). Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Personnel should not enter or leave the facility through the air locks, except in an emergency.
2. In a Class III cabinet operation, personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by personnel entering the facility. When exiting, personnel remove laboratory clothing in the inner change room before entering the shower area. Soiled clothing is sterilized in an autoclave.
3. In an ABSL-4 suit operation, a complete clothing change is required. A personal shower is required following removal of the decontaminated suit. Soiled lab clothing is autoclaved before laundering.

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 4

4. Supplies and materials are introduced into the facility via a double-door autoclave or fumigation chamber. After the outer door is secure, personnel inside the facility open the inner door to retrieve the materials. 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 “sterilization cycle” or the fumigation chamber has been decontaminated.
5. A system is established for the reporting of accidents, incidents, exposures, and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such a reporting/surveillance system is the availability of a facility for the quarantine, isolation, and medical care of persons with potential or known laboratory-associated illnesses.
6. The serum samples collected are analyzed at intervals. The results are communicated to the participants.

C. Safety Equipment (Primary Barriers)

1. Laboratory animals infected with Biosafety Level 4 agents must be housed within a Class III biological safety cabinet in a BSL-4 Cabinet Laboratory. In a BSL-4 Suit Laboratory, all personnel are required to wear one-piece positive pressure suits ventilated with a life support system. Infected animals should be housed in a partial 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).
2. The use of disposable material that does not require cleaning, including animal caging, should be considered. Disposable materials must be autoclaved on exit from the facility and then incinerated.

Vertebrate Animal Biosafety Level Criteria — Animal Biosafety Level 4

D. Facilities (Secondary Barriers)

BSL-4 animal areas may be included as an integral part of BSL-4 Cabinet Laboratories or Suit Laboratories as described in Section III of this document. The facility requirements described in the BSL-4 Laboratory section should be utilized in conjunction with the caging described in the equipment section above.

References:

1. Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 1996
2. U.S. Department of Agriculture. Laboratory Animal Welfare Regulations - 9 CFR, Subchapter A, Parts 1, 2, and 3.
3. Occupational Health and Safety in the Care of Research Animals. National Academy Press, Washington, D.C., 1996.
4. Subcommittee on Arbovirus Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates. 1980. Am J Trop Med Hyg **29**(6):1359-1381.
5. Centers for Disease Control and Prevention. 1998. Fatal Cercopithecine herpesvirus 1 (B Virus) Infection Following a Mucocutaneous Exposure and Interim Recommendations for Worker Protection. MMWR **47**(49); 1073-6,1083
6. Guide for the Care and Use of Laboratory Animals (1)
7. Occupational Health and Safety in the Care of Research Animals (3)
8. Centers for Disease Control and Prevention. 1998. (5)
9. Occupational Health and Safety in the Care of Research Animals (3)
10. Occupational Health and Safety in the Care of Research Animals. (3)

Table 1. Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals Are Used.

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy human adults.	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species.	Standard animal facility <ul style="list-style-type: none"> ● No recirculation of exhaust air ● Directional air flow recommended ● Handwashing sink recommended
2	Associated with human disease. Hazard: percutaneous exposure, ingestion, mucous membrane exposure.	ABSL-1 practices plus: <ul style="list-style-type: none"> ● 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 facility plus: <ul style="list-style-type: none"> ● Autoclave available ● Handwashing sink available in the animal room. ● Mechanical cage washer used
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects.	ABSL-2 practices plus: <ul style="list-style-type: none"> ● Controlled access ● Decontamination of clothing before laundering ● Cages decontaminated before bedding removed ● Disinfectant foot bath as needed 	ABSL-2 equipment plus: <ul style="list-style-type: none"> ● Containment equipment for housing animals and cage dumping activities ● Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: appropriate respiratory protection 	ABSL-2 facility plus: <ul style="list-style-type: none"> ● 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: <ul style="list-style-type: none"> ● 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: <ul style="list-style-type: none"> ● 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: <ul style="list-style-type: none"> ● Separate building or isolated zone ● Dedicated supply and exhaust, vacuum and decontamination systems ● Other requirements outlined in the text

SECTION V

Risk Assessment

“Risk” implies the probability that harm, injury, or disease will occur. In the context of the microbiological and biomedical laboratories, the assessment of risk focuses primarily on the prevention of laboratory-associated infections. When addressing laboratory activities involving infectious or potentially infectious material, risk assessment is a critical and productive exercise. It helps to assign the biosafety levels (facilities, equipment, and practices) that reduce the worker’s and the environment’s risk of exposure to an agent to an absolute minimum. The intent of this section is to provide guidance and to establish a framework for selecting the appropriate biosafety level.

Risk assessment can be qualitative or quantitative. In the presence of known hazards (e.g., residual levels of formaldehyde gas after a laboratory decontamination), quantitative assessments can be done. But in many cases, quantitative data will be incomplete or even absent (e.g., investigation of an unknown agent or receipt of an unlabeled sample). Types, subtypes, and variants of infectious agents involving different or unusual vectors, the difficulty of assays to measure an agent’s amplification potential, and the unique considerations of genetic recombinants are but a few of the challenges to the safe conduct of laboratory work. In the face of such complexity, meaningful quantitative sampling methods are frequently unavailable. Therefore, the process of doing a risk assessment for work with biohazardous materials cannot depend on a prescribed algorithm.

The laboratory director or principal investigator is responsible for assessing risks in order to set the biosafety level for the work. This should be done in close collaboration with the Institutional Biosafety Committee (and/or other biosafety professionals as needed) to ensure compliance with established guidelines and regulations.

In performing a qualitative risk assessment, all the risk factors are first identified and explored. There may be related information available, such as this manual, the NIH Recombinant DNA

Risk Assessment

Guidelines, the Canadian Laboratory Biosafety Guidelines, or the WHO Biosafety Guidelines. In some cases, one must rely on other sources of information such as field data from subject matter experts. This information is interpreted for its tendency to raise or lower the risk of laboratory-acquired infection.¹

The challenge of risk assessment lies in those cases where complete information on these factors is unavailable. A conservative approach is generally advisable when insufficient information forces subjective judgement. Universal precautions are always advisable.

The factors of interest in a risk assessment include:

- The *pathogenicity* of the infectious or suspected infectious agent, including disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease). The more severe the potentially acquired disease, the higher the risk. For example, *staphylococcus aureus* only rarely causes a severe or life threatening disease in a laboratory situation and is relegated to BSL-2. Viruses such as Ebola, Marburg, and Lassa fever, which cause diseases with high mortality rates and for which there are no vaccines or treatment, are worked with at BSL-4. However, disease severity needs to be tempered by other factors. Work with human immunodeficiency virus (HIV) and hepatitis B virus is also done at BSL-2, although they can cause potentially lethal disease. But they are not transmitted by the aerosol route, the incidence of laboratory-acquired infection is extremely low for HIV, and an effective vaccine is available for hepatitis B.
- The *route of transmission* (e.g., parenteral, airborne, or by ingestion) of newly isolated agents may not be definitively established. Agents that can be transmitted by the aerosol route have caused most laboratory infections. It is wise, when planning work with a relatively uncharacterized agent with an uncertain mode of transmission, to consider the *potential for aerosol*

Risk Assessment

transmission. The greater the aerosol potential, the higher the risk.

- *Agent stability* is a consideration that involves not only aerosol infectivity (e.g., from spore-forming bacteria), but also the agent's ability to survive over time in the environment. Factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfectants must be considered.
- The *infectious dose* of the agent is another factor to consider. Infectious dose can vary from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized laboratory worker, and may pose a serious risk to those with lesser resistance. The laboratory worker's *immune status* is directly related to his/her susceptibility to disease when working with an infectious agent.
- The *concentration* (number of infectious organisms per unit volume) will be important in determining the risk. Such a determination will include consideration of the milieu containing the organism (e.g., solid tissue, viscous blood or sputum, or liquid medium) and the laboratory activity planned (e.g., agent amplification, sonication, or centrifugation). The volume of concentrated material being handled is also important. In most instances, the risk factors increase as the working volume of high-titered microorganisms increases, since additional handling of the materials is often required.
- The *origin* of the potentially infectious material is also critical in doing a risk assessment. "Origin" may refer to geographic location (e.g., domestic or foreign); host (e.g., infected or uninfected human or animal); or nature of source (potential zoonotic or associated with a disease outbreak). From another perspective, this factor can also consider the potential of agents to endanger American livestock and poultry.

Risk Assessment

- The *availability of data from animal studies*, in the absence of human data, may provide useful information in a risk assessment. Information about pathogenicity, infectivity, and route of transmission in animals may provide valuable clues. Caution must always be exercised, however, in translating infectivity data from one species of animal to another species.
- The established *availability of an effective prophylaxis* or therapeutic intervention is another essential factor to be considered. The most common form of prophylaxis is immunization with an effective vaccine. Risk assessment includes determining the availability of effective immunizations. In some instances, immunization may affect the biosafety level (e.g., the BSL-4 Junin virus can be worked on at BSL-3 by an immunized worker). Immunization may also be passive (e.g., the use of serum immunoglobulin in HBV exposures). However important, immunization only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Occasionally, immunization or therapeutic intervention (antibiotic or antiviral therapy) may be particularly important in field conditions. The offer of immunizations is part of risk management.
- *Medical surveillance* ensures that the safeguards decided upon in fact produce the expected health outcomes. Medical surveillance is part of risk management. It may include serum banking, monitoring employee health status, and participating in post-exposure management.
- Risk assessment must also include an evaluation of the *experience and skill level of at-risk personnel* such as laboratorians and maintenance, housekeeping, and animal care personnel (see Section III). Additional education may be necessary to ensure the safety of persons working at each biosafety level.

Risk Assessment

The infectious agents whose risk is evaluated often will fall into the following discrete categories.

Materials containing known infectious agents. The characteristics of most known infectious agents have been well identified. Information useful to risk assessment can be obtained from laboratory investigations, disease surveillance, and epidemiological studies. Infectious agents known to have caused laboratory-associated infections are included in this volume's agent summary statements (see Section VII). Other sources include the American Public Health Association's manual, *Control of Communicable Diseases*.² Literature reviews on laboratory acquired infections also may be helpful.^{3,4,5,6,7,8}

Materials containing unknown infectious agents. The challenge here is to establish the most appropriate biosafety level with the limited information available. Often these are clinical specimens. Some questions that may help in this risk assessment include:

1. Why is an infectious agent suspected?
2. What epidemiological data are available? What route of transmission is indicated? What is the morbidity or mortality rate associated with the agent?
3. What medical data are available?

The responses to these questions may identify the agent or a surrogate agent whose existing agent summary statement can be used to determine a biosafety level. In the absence of hard data, a conservative approach is advisable.

Materials containing recombinant DNA molecules. This category of agents includes microorganisms that have been genetically modified through recombinant DNA technologies. These technologies continue to evolve rapidly. Experimental procedures designed to derive novel recombinant viruses, bacteria, yeast, and other microorganisms have become commonplace in recent years. It is highly likely that future applications of recombinant DNA technology will produce new hybrid viruses. The National Institutes of Health publication, *Guidelines for Research Involving Recombinant DNA Molecules*,⁹

Risk Assessment

is a key reference in establishing an appropriate biosafety level for work involving recombinant microorganisms.

In selecting an appropriate biosafety level for such work, perhaps the greatest challenge is to evaluate the potential increased biohazard associated with a particular genetic modification. In most such cases, the selection of an appropriate biosafety level begins by establishing the classification of the non-modified virus. Among the recombinant viruses now routinely developed are adenoviruses, alphaviruses, retroviruses, vaccinia viruses, herpesviruses, and others designed to express heterologous gene products. However, the nature of the genetic modification and the quantity of virus must be carefully considered when selecting the appropriate biosafety level for work with a recombinant virus.

Among the points to consider in work with recombinant microorganisms are:

- Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?
- Does the modification have the potential to alter the host range or cell tropism of the virus?
- Does the modification have the potential to increase the replication capacity of the virus?
- Does the inserted gene encode a known oncogene?
- Does the inserted gene have the potential for altering the cell cycle?
- Does the viral DNA integrate into the host genome?
- What is the probability of generating replication-competent viruses?

This list of questions is not meant to be inclusive. Rather, it serves as an example of the information needed to judge whether a higher biosafety level is needed in work with genetically modified microorganisms. Since in many cases the answers to the above questions will not be definitive, it is important that the organization have a properly constituted and informed Institutional Biosafety Committee, as outlined in the NIH guidelines, to evaluate the risk assessment.

Risk Assessment

Materials that may or may not contain unknown infectious agents. In the absence of information that suggests an infectious agent, universal precautions are indicated.

Animal studies. Laboratory studies involving animals may present many different kinds of physical, environmental, and biological hazards. The specific hazards present in any particular animal facility are unique, varying according to the species involved and the nature of the research activity. The risk assessment for biological hazard should particularly focus on the animal facility's potential for increased exposure, both to human pathogens and to zoonotic agents.

The animals themselves can introduce new biological hazards to the facility. Latent infections are most common in field-captured animals or in animals coming from unscreened herds. For example, monkey B-virus presents a latent risk to individuals who handle macaques. The animal routes of transmission must also be considered in the risk assessment. Animals that shed virus through respiratory dissemination or dissemination in urine or feces are far more hazardous than those that do not. Animal handlers in research facilities working on infectious agents have a greater risk of exposure from the animals' aerosols, bites, and scratches. Section IV describes the practices and facilities applicable to work on animals infected with agents assigned to corresponding Biosafety Levels 1-4.¹

Other applications. The described risk assessment process is also applicable to laboratory operations other than those involving the use of primary agents of human disease. It is true that microbiological studies of animal host-specific pathogens, soil, water, food, feeds, and other natural or manufactured materials, pose comparatively lower risks for the laboratory worker. Nonetheless, microbiologists and other scientists working with such materials may find the practices, containment equipment, and facility recommendations described in this publication of value in developing operational standards to meet their own assessed needs.

Risk Assessment

References:

1. Knudsen, R.C. 1998. Risk Assessment for Biological Agents in the Laboratory. In J. Y. Richmond, Ph.D, R.B.P. (ed.) Rational Basis for Biocontainment: Proceedings of the Fifth National Symposium on Biosafety. American Biological Safety Association, Mundelein, IL.
2. Benenson, Abram S., Editor. Control of Communicable Diseases Manual. 16th Edition, 1995. American Public Health Association, Washington, D.C. 20005.
3. Collins, C.H. Laboratory-acquired infections, history, incidence, causes and prevention. Butterworths, and Co. Ltd. 1983.
4. Richmond, Jonathan Y., and McKinney, Robert W., Editors. Biosafety in Microbiological and Biomedical Laboratories. Public Health Service, 3rd Edition, May, 1993.
5. Sewell, David L. Laboratory Associated Infections and Biosafety. Clinical Microbiology Reviews, 8:389-405, 1995
6. Sulkin, S.E., Pike, R.M. 1949. Viral Infections contracted in the laboratory. New England J. Medicine. 241:205-213.
7. Sulkin, S.E., Pike, R.M. 1951. Survey of Laboratory acquired infections. Am J Public health 41:769-781.
8. Sullivan, J.F. Songer, J.R., Estrem, I.E. 1978. Laboratory acquired infections at the National Animal Disease Center, 1960-1975. Health Lab Sci 15: 58-64.
9. National Institutes of Health. Guidelines for Research Involving Recombinant DNA Molecules. (Washington: GPO, 1998) Federal Register. 59FR34496.

Other Resources:

- NIH Guidelines for Recombinant DNA Molecules:
<http://www.NIH.gov/od/orda/toc.html>
- NIH Office of Recombinant DNA Activities:
<http://www.NIH.gov/od/orda>

SECTION VI

Recommended Biosafety Levels for Infectious Agents and Infected Animals

Selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a number of factors (see Section V, Risk Assessment). Some of the most important are: the virulence, pathogenicity, biological stability, route of spread, and communicability of the agent; the nature or function of the laboratory; the procedures and manipulations involving the agent; the endemicity of the agent; and the availability of effective vaccines or therapeutic measures.

Agent summary statements in this section provide guidance for the selection of appropriate biosafety levels. Specific information on laboratory hazards associated with a particular agent, and recommendations regarding practical safeguards that can significantly reduce the risk of laboratory-associated diseases, are included. Agent summary statements are presented for agents which meet one or more of the following criteria: the agent is a proven hazard to laboratory personnel working with infectious materials (e.g., hepatitis B virus, *M. tuberculosis*); the potential for laboratory-associated infections is high, even in the absence of previously documented laboratory-associated infections (e.g., exotic arboviruses); or the consequences of infection are grave.

Recommendations for the use of vaccines and toxoids are included in agent summary statements when such products are available, either as licensed or Investigational New Drug (IND) products (see Appendix B, Immunoprophylaxis). When applicable, recommendations for the use of these products are based on current recommendations of the Public Health Service Advisory Committee on Immunization Practice, and are specifically targeted to at-risk laboratory personnel and others who must work in or enter laboratory areas. These specific recommendations should in no way preclude the routine use of such products as diphtheria-tetanus toxoids, poliovirus vaccine, influenza vaccine and others, because of the potential risk of community exposures irrespective of any laboratory risks. Appropriate precautions should be taken in the administration of live

Recommended Biosafety Levels For Infectious Agents and Infected Animals

attenuated virus vaccines in individuals with altered immunocompetence or other medical condition (e.g., pregnancy), in which a viral infection could result in adverse consequences.

Risk assessments and biosafety levels recommended in the agent summary statements presuppose a population of immunocompetent individuals. Persons with altered immunocompetence may be at an increased risk when exposed to infectious agents. Immunodeficiency may be hereditary, congenital, or induced by a number of neoplastic or infectious diseases, by therapy, or by radiation. The risk of becoming infected or the consequence of infection may also be influenced by such factors as age, sex, race, pregnancy, surgery (e.g., splenectomy, gastrectomy), predisposing diseases (e.g., diabetes, lupus erythematosus) or altered physiological function. These and other variables must be considered in applying the generic risk assessments of the agent summary statements to specific activities of selected individuals.

The biosafety level assigned to an agent is based on the activities typically associated with the growth and manipulation of the quantities and concentrations of infectious agents required to accomplish identification or typing. If activities with clinical materials pose a lesser risk to personnel than those activities associated with manipulation of cultures, a lower biosafety level is recommended. On the other hand, if the activities involve large volumes and/or concentrated preparations ("production quantities"), or manipulations which are likely to produce aerosols or which are otherwise intrinsically hazardous, additional personnel precautions and increased levels of primary and secondary containment may be indicated.

"Production quantities" refers to large volumes or concentrations of infectious agents considerably in excess of those typically used for identification and typing activities. Propagation and concentration of infectious agents, as occurs in large-scale fermentations, antigen and vaccine production, and a variety of other commercial and research activities, clearly deal with significant masses of infectious agents that are reasonably considered "production quantities." However, in terms of potentially increased risk as a function of the mass of infectious agents, it is not

Recommended Biosafety Levels For Infectious Agents and Infected Animals

possible to define "production quantities" in finite volumes or concentrations for any given agent. Therefore, the laboratory director must make an assessment of the activities conducted and select practices, containment equipment, and facilities appropriate to the risk, irrespective of the volume or concentration of agent involved.

Occasions will arise when the laboratory director should select a biosafety level higher than that recommended. For example, a higher biosafety level may be indicated by the unique nature of the proposed activity (e.g., the need for special containment for experimentally generated aerosols for inhalation studies) or by the proximity of the laboratory to areas of special concern (e.g., a diagnostic laboratory located near patient care areas). Similarly, a recommended biosafety level may be adapted to compensate for the absence of certain recommended safeguards. For example, in those situations where Biosafety Level 3 is recommended, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations, provided the recommended standard microbiological practices, special practices, and safety equipment for Biosafety Level 3 are rigorously followed.

One example involves work with the Human Immunodeficiency Virus (HIV). Routine diagnostic work with clinical specimens can be done safely at Biosafety Level 2, using Biosafety Level 2 practices and procedures. Research work (including co-cultivation, virus replication studies, or manipulations involving concentrated virus) can be done in a BSL-2 facility, using BSL-3 practices and procedures. Virus production activities, including virus concentrations, require a BSL-3 facility and use of BSL-3 practices and procedures (see Agent Summary Statement).

The decision to adapt Biosafety Level 3 recommendations in this manner should be made only by the laboratory director. This adaptation, however, is not suggested for agent production operations or activities where procedures are frequently changing. The laboratory director should also give special consideration to

Recommended Biosafety Levels For Infectious Agents and Infected Animals

selecting appropriate safeguards for materials that may contain a suspected agent. For example, sera of human origin may contain hepatitis B virus, and therefore, all blood or blood-derived fluids should be handled under conditions which reasonably preclude cutaneous, mucous membrane or parenteral exposure of personnel. Sputa submitted to the laboratory for tubercle bacilli assay should be handled under conditions which reasonably preclude the generation of aerosols during the manipulation of clinical materials or cultures.

The infectious agents that meet the previously stated criteria are listed by category of agent in Section VII. To use these summaries, first locate the agent in the listing under the appropriate category of agent. Second, utilize the practices, safety equipment, and type of facilities recommended in the agent summary statement as described in Section VII for working with clinical materials, cultures or infectious agents, or infected animals.

The laboratory director is also responsible for appropriate risk assessment and for utilization of appropriate practices, containment equipment, and facilities for agents not included in the agent summary statements.

SECTION VII

Agent Summary Statements

- **Section VII-A: Bacterial Agents**

Agent: *Bacillus anthracis*

Numerous cases of laboratory-associated anthrax, occurring primarily at facilities conducting anthrax research, have been reported.^{1,2} No laboratory-associated cases of anthrax have been reported in the United States since the late 1950s when human anthrax vaccine was introduced. Any work with *B. anthracis* requires special security considerations due to its potential use for purposes of biological terrorism. Naturally and experimentally infected animals pose a potential risk to laboratory and animal care personnel.

Laboratory Hazards: The agent may be present in blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and rarely, in urine and feces. Direct and indirect contact of the intact and broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation, and rarely, exposure to infectious aerosols are the primary hazards to laboratory personnel.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for studies utilizing experimentally infected laboratory rodents. Biosafety Level 3 practices, containment equipment, and facilities are recommended for work involving production quantities or concentrations of cultures, and for activities with a high potential for aerosol production.

Note: A licensed vaccine is available through the Centers for Disease Control and Prevention; however, immunization of laboratory personnel is not recommended unless frequent work with clinical specimens or diagnostic cultures is anticipated (e.g.,

Agent Summary Statements – Bacterial Agents

animal disease diagnostic laboratory). In these facilities immunization is recommended for all persons working with the agent, all persons working in the same laboratory room where the cultures are handled, and persons working with infected animals.

Transfer of Agent: For a permit to import this agent, contact CDC. Contact the Department of Commerce for a permit to export this agent. Laboratory registration with CDC is required before sending or receiving this select agent. An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Agent: *Bordetella pertussis*

Bordetella pertussis, a human respiratory pathogen of worldwide distribution, is the causative agent of whooping cough. The disease is typically a childhood illness; however, the agent has increasingly been associated with adult illness.^{3,4,5} Several outbreaks in health-care workers have been reported in the literature.^{6,7} Adolescents and adults with atypical or undiagnosed disease can serve as reservoirs of infection and transmit the organism to infants and children.⁸ Eight cases of infection with *B. pertussis* in adults have been documented at a large research institution. The individuals involved did not work directly with the organism, but had access to common laboratory spaces where the organism was manipulated. One case of secondary transmission to a family member was documented.⁹ A similar incident occurred at a large Midwestern university resulting in two documented cases of laboratory-acquired infection and one documented case of secondary transmission.¹⁰ Other laboratory-acquired infections with *B. pertussis* have been reported, as well as adult-to-adult transmission in the workplace.^{11, 12} Laboratory-acquired infections resulting from the manipulation of clinical specimens or isolates have not been reported. The attack rate of this airborne infection is influenced by intimacy and frequency of exposure of susceptible individuals.

Laboratory Hazards: The agent may be present in respiratory secretions, but is not found in blood or tissue. Since the natural mode of transmission is by the respiratory route, the greatest

Agent Summary Statements – Bacterial Agents

potential hazard is aerosol generation during the manipulation of cultures or concentrated suspensions of the organism.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical materials or cultures. Animal Biosafety Level 2 should be used for the housing of infected animals. Primary containment devices and equipment (e.g., biological safety cabinets, centrifuge safety cups, or specially designed safety centrifuges) should be used for activities likely to generate potentially infectious aerosols. Biosafety Level 3 practices, procedures, and facilities are appropriate when engaged in large scale production operations.

Note: Pertussis vaccines are available but are not currently recommended for use in adults. 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 (MMWR) for recommendations for pertussis vaccination in adults.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Brucella* (*B. abortus*, *B. canis*, *B. melitensis*, *B. suis*)

Brucellosis continues to be the most commonly reported laboratory-associated bacterial infection.^{13,14,15} *B. abortus*, *B. canis*, *B. melitensis*, and *B. suis* have all caused illness in laboratory personnel.^{16,17,18} Hypersensitivity to *Brucella* antigens is also a hazard to laboratory personnel. Occasional cases have been attributed to exposure to experimentally and naturally infected animals or their tissues.

Laboratory Hazards: The agent may be present in blood, cerebrospinal fluid, semen, and occasionally urine. Most laboratory-associated cases have occurred in research facilities and have involved exposure to *Brucella* organisms grown in large quantities. Cases have also occurred in the clinical laboratory set-

Agent Summary Statements – Bacterial Agents

ting from sniffing bacteriological cultures.¹⁹ Direct skin contact with cultures or with infectious clinical specimens from animals (e.g., blood, uterine discharges) are commonly implicated in these cases. Aerosols generated during laboratory procedures have caused large outbreaks.^{20,21} Mouth pipetting, accidental parenteral inoculations, and sprays into eyes, nose, and mouth have also resulted in infection.

Recommended Precautions: Biosafety Level 2 practices are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp. Biosafety Level 3 and Animal Biosafety Level 3 practices, containment equipment, and facilities are recommended, respectively, for all manipulations of cultures of the pathogenic *Brucella* spp. listed in this summary, and for experimental animal studies.

Note: While human *Brucella* vaccines have been developed and tested in other countries with limited success, at the time of this publication no human vaccine is available in the United States.²²

Transfer of Agent: For a permit to import this agent, contact CDC. Contact the Department of Commerce for a permit to export this agent. Laboratory registration with CDC is required before sending or receiving this select agent. An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Agent: *Burkholderia pseudomallei* (*Pseudomonas pseudomallei*)

Two laboratory-associated cases of melioidosis have been reported: one associated with a massive aerosol and skin exposure;²³ the second resulting from an aerosol created during the open-flask sonication of a culture presumed to be *Ps. cepacia*.²⁴

Laboratory Hazards: The agent may be present in sputum, blood, wound exudates and various tissues depending on the infection's site of localization. Direct contact with cultures and

Agent Summary Statements – Bacterial Agents

infectious materials from humans, animals, or the environment, ingestion, autoinoculation, and exposure to infectious aerosols and droplets are the primary laboratory hazards. The agent has been demonstrated in blood, sputum, and abscess materials and may be present in soil and water samples from endemic areas.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids, tissues, and cultures. Gloves should be worn when handling infected animals, during their necropsy, and when there is the likelihood of direct skin contact with infectious materials. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with a high potential for aerosol or droplet production, and for activities involving production quantities or concentrations of infectious materials. Vaccines are not currently available for use in humans.

Transfer of Agent: Contact the Department of Commerce for a permit to export this agent. For a permit to import this agent, contact the CDC. Laboratory registration with CDC is required before sending and receiving this agent.

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

C. jejuni/*C. coli gastroenteritis* is rarely a cause of laboratory-associated illness, although laboratory-acquired cases have been documented.^{25,26,27} 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. Experimentally infected animals are also a potential source of infection.²⁸

Laboratory Hazards: Pathogenic campylobacters may occur in fecal specimens in large numbers. *C. fetus* subsp. *fetus* may also be present in blood, exudates from abscesses, tissues, and sputa. Ingestion or parenteral inoculation of *C. jejuni* constitute the primary laboratory hazards. The oral ingestion of 500 organ-

Agent Summary Statements – Bacterial Agents

isms caused infection in one individual.²⁹ The importance of aerosol exposure is not known.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals. Vaccines are currently not available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Chlamydia psittaci*, *C. pneumoniae*, *C. trachomatis*

Psittacosis, lymphogranuloma venereum (LGV), and trachoma infections were at one time among the most commonly reported laboratory-associated bacterial infections.³⁰ In cases reported before 1955,³¹ the majority of infections were psittacosis, and these had the highest case fatality rate of laboratory-acquired infectious agents. Contact with and exposure to infectious aerosols in the handling, care, or necropsy of naturally or experimentally infected birds are the major sources of laboratory-associated psittacosis. Infected mice and eggs are less important sources of *C. psittaci*. Laboratory animals are not a reported source of human infection with *C. trachomatis*.

Laboratory Hazards: *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 psittacosis. The primary laboratory hazards of *C. trachomatis* 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

Agent Summary Statements – Bacterial Agents

eggs. Infectious aerosols may also pose a potential source of infection.

Recommended Precautions: Biosafety Level 2 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 potentially infected with *C. psittaci* or *C. trachomatis*. 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. Animal Biosafety Level 2 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds. 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. Biosafety Level 3 facilities and practices are indicated for activities with high potential for droplet or aerosol production and for activities involving large quantities or concentrations of infectious materials.

Note: Vaccines are not currently available for use in humans.

Transfer of Agent: Contact the Department of Commerce for a permit to export these agents.

Agent: *Clostridium botulinum*

While there is only one report³² of botulism associated with the handling of the agent or toxin in the laboratory or working with naturally or experimentally infected animals, the consequences of such intoxications must still be considered quite grave. Work with cultures of *C. botulinum* requires special security considerations due to their potential use for purposes of biological terrorism.

Laboratory Hazards: *C. botulinum* or its toxin may be present in a variety of food products, clinical materials (serum, feces), and environmental samples (soil, surface water). Exposure to the toxin of *C. botulinum* is the primary laboratory hazard. The toxin

Agent Summary Statements – Bacterial Agents

may be absorbed after ingestion or following contact with the skin, eyes, or mucous membranes, including the respiratory tract.³³ Accidental parenteral inoculation may also represent a significant exposure to toxin. Broth cultures grown under conditions of optimal toxin production may contain 2×10^6 mouse LD₅₀ per mL.³⁴

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities with materials known to contain or potentially containing the toxin. A pentavalent (ABCDE) botulism toxoid is available through the Centers for Disease Control and Prevention, as an investigational new drug (IND). This toxoid is recommended for personnel working with cultures of *C. botulinum* or its toxins. Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontaminating work surfaces and spills of cultures or toxin. Additional primary containment and personnel precautions, such as those recommended for Biosafety Level 3, are indicated for activities with a high potential for aerosol or droplet production, and those involving production quantities of toxin. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.

Transfer of Agent: For a permit to import this agent, contact CDC. Laboratory registration with CDC is required before sending or receiving this select agent.

Agent: Clostridium tetani

Although the risk of infection to laboratory personnel is negligible, five incidents related to exposure of personnel during manipulation of the toxin have been recorded.³⁵

Laboratory Hazards: Accidental parenteral inoculation and ingestion of the toxin are the primary hazards 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.

Agent Summary Statements – Bacterial Agents

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities involving the manipulation of cultures 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.³⁶ 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 (MMWR) for recommendations for adult vaccination against *C. tetani*.

Transfer of Agent: For a permit to import this agent, contact CDC. Contact the Department of Commerce for a permit to export this agent.

Agent: *Corynebacterium diphtheriae*

Laboratory-associated infections with *C. diphtheriae* have been documented. Laboratory animal-associated infections have not been reported.³⁷

Laboratory Hazards: The agent may be present in exudates or secretions of the nose, throat (tonsil), pharynx, larynx, wounds, in blood, and on the skin. Inhalation, accidental parenteral inoculation, and ingestion are the primary laboratory hazards.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. Animal Biosafety Level 2 facilities are recommended for studies utilizing infected laboratory animals. 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 toxin exposures and work with infectious materials to laboratory and animal care personnel.³⁸ The reader is advised to consult the current recommendations of the Advisory Committee on Immunization Practices (ACIP) published in the

Agent Summary Statements – Bacterial Agents

CDC Morbidity and Mortality Weekly Report (MMWR) for recommendations for vaccination against *C. diphtheriae*.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Escherichia coli* (Cytotoxin-producing (VTEC/SLT) organisms)

Cytotoxin-producing (VTEC/SLT) strains of *Escherichia coli* (also called enterohemorrhagic strains) are a demonstrated hazard to laboratory personnel in the United States and elsewhere.^{39,40,41} Hemolytic uremic syndrome occurs in a small proportion of patients (usually children) and is responsible for most deaths associated with infections with these organisms. Domestic farm animals (particularly bovines) are significant reservoirs of the organisms. However, experimentally infected small animals are also sources of infection in the laboratory.

Laboratory Hazards: Enterohemorrhagic *E. coli* is usually isolated from feces. A variety of foods contaminated with the organisms may serve as vehicles of spread, and include uncooked ground beef and unpasteurized dairy products. It may rarely be found in blood of infected humans or animals. Ingestion is the primary laboratory hazard. The importance of aerosol exposure is not known.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Animal Biosafety Level 2 facilities and practices are recommended for activities with experimentally or naturally infected animals. Vaccines are currently not available for use in humans. The reader is advised to consult the current related recommendations of the ACIP published in the CDC *Morbidity and Mortality Weekly Report* (MMWR) for the existence of recommendations for vaccination against enterohemorrhagic strains of *E. coli*.

Agent Summary Statements – Bacterial Agents

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Francisella tularensis*

Tularemia has been a commonly reported laboratory-associated bacterial infection.⁴² Almost all cases occurred at facilities involved in tularemia research. Occasional cases have been related to work with naturally or experimentally infected animals or their ectoparasites. Although not reported, cases have occurred in clinical laboratories. Work with cultures of *F. tularensis* requires special security considerations due to their potential use for purposes of biological terrorism.

Laboratory Hazards: The agent may be present in lesion exudates, respiratory secretions, cerebrospinal fluid, blood, urine, tissues 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 have resulted in infection. Infection has been more commonly associated with cultures than with clinical materials and infected animals. The human 25% to 50% infectious dose is approximately 10 organisms by the respiratory route.⁴³

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials of human or animal origin containing or potentially containing *Francisella tularensis*. Biosafety Level 3 and Animal Biosafety Level 3 practices, containment equipment, and facilities are recommended, respectively, for all manipulations of cultures and for experimental animal studies.

Note: Vaccination for *F. tularensis* is available and should be considered for personnel working with infectious materials or infected rodents. Vaccination is recommended for persons working with the agent or infected animals, and for persons working in or entering the laboratory or animal room where cultures or infected animals are maintained.⁴⁴ The reader is advised to consult the current recommendations of the Advisory

Agent Summary Statements – Bacterial Agents

Committee on Immunization Practices (ACIP) published in the CDC Morbidity and Mortality Weekly Report (MMWR) for recommendations for vaccination against *F. tularensis*.

Transfer of Agent: For a permit to import this agent, contact CDC. Contact the Department of Commerce for a permit to export this agent. Laboratory registration with CDC is required before sending or receiving this select agent.

Agent: *Helicobacter pylori*

Since its discovery in 1982, *Helicobacter pylori* has received increasing attention as an agent of gastritis.⁴⁵ The main habitat of *H. pylori* is the human gastric mucosa. Human infection with *H. pylori* may be long in duration with few or no symptoms, or may present as an acute gastric illness. Both experimental and accidental laboratory-acquired human infections with *H. pylori* have been reported.^{46,47} The agent may be present in gastric or oral secretions and stool. Transmission, while incompletely understood, is thought to be by the fecal-oral or oral-oral route.

Laboratory Hazards: The agent may be present in gastric and oral secretions and stool. Ingestion is the primary known laboratory hazard. The importance of aerosol exposures is unknown.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially containing the agents. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals. Vaccines are currently not available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Leptospira interrogans* - all serovars

Agent Summary Statements – Bacterial Agents

Leptospirosis is a well-documented laboratory hazard. Pike reported 67 laboratory-associated infections and 10 deaths,⁴⁸ and three additional cases have been reported elsewhere.⁴⁹

An experimentally infected rabbit was identified as the source of an infection with *L. interrogans* serovar *icterohemorrhagiae*.⁵⁰ Direct and indirect contact with fluids and tissues of experimentally or naturally infected mammals during handling, care, or necropsy is a potential source of infection. In animals with chronic kidney infections, the agent is shed in the urine in enormous numbers for long periods of time.

Laboratory Hazards: The agent 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 with cultures or infected tissues or body fluids—especially urine—are the primary laboratory hazards. The importance of aerosol exposure is not known.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious tissues, body fluids, and cultures, and for the housing of infected animals. Gloves are recommended for the handling and necropsy of infected animals, and when there is the likelihood of direct skin contact with infectious materials. Vaccines are not currently available for use in humans.

Transfer of Agent: For a permit to import these agents, contact CDC. An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Agent: *Listeria monocytogenes*

Listeria monocytogenes poses a potential hazard to laboratory personnel. The gram-positive, non-spore-forming, aerobic bacilli are hemolytic and catalase-positive.⁵¹ Bacteria have been isolated from soil, dust, human food, animals, and asymptomatic humans.^{52,53} Most cases of listeriosis have arisen from eating contaminated food products, most notably soft

Agent Summary Statements – Bacterial Agents

cheeses, raw meat, and unwashed raw vegetables.⁵⁴ Although healthy adults and children can contract a *Listeria* infection, they do not usually become seriously ill. At risk of severe illness are pregnant women, newborns, and persons with impaired immune function.

Laboratory Hazards: *Listeria monocytogenes* may be found in feces, CSF, and blood, as well as food and environmental materials.^{55,56} Naturally or experimentally infected animals are a source of exposure to laboratory workers and animal care personnel, and other animals. Ingestion is the most likely mode of exposure, but *Listeria* can also cause eye and skin infections following a direct exposure. *Listeria monocytogenes* infections in pregnant women occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus and may result in disseminated abscesses contributing to a mortality rate of nearly 100%.⁵⁷

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn while handling infected cultures. Animal Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with experimentally or naturally infected animals. Vaccines are not currently available for use in humans.⁵⁸ Pregnant women who work with *Listeria monocytogenes* in the clinical or research laboratory setting should be fully informed of the potential hazards associated with the organism, including potential risks to the fetus.

Transfer of Agent: An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Agent: *Legionella pneumophila*; other *Legionella*-like agents

A single documented nonfatal laboratory-associated case of legionellosis, due to presumed aerosol or droplet exposure during animal challenge studies with Pontiac Fever agent (*L. pneumo-*

Agent Summary Statements – Bacterial Agents

phila), has been recorded.⁵⁹ Human-to-human spread has not been documented.

Experimental infections are readily produced in guinea pigs and embryonate chicken eggs.⁶⁰ Challenged rabbits develop antibodies but not clinical disease. Mice are refractory to parenteral exposure. Unpublished studies at the Centers for Disease Control and Prevention have shown that animal-to-animal transmission did not occur in a variety of experimentally infected mammalian and avian species.

Laboratory Hazards: The agent may be present in pleural fluid, tissue, sputum, and environmental sources (e.g., cooling tower water). Because the natural mode of transmission appears to be airborne, the greatest potential hazard is the generation of aerosols during the manipulation of cultures or of other materials containing high concentrations of infectious microorganisms (e.g., infected yolk sacs and tissues).

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical materials or cultures, and for the housing of infected animals. Biosafety Level 3 practices with primary containment devices and equipment (e.g., biological safety cabinets, centrifuge safety cups) are used for activities likely to generate potentially infectious aerosols and for activities involving production quantities of microorganisms.

Note: Vaccines are not currently available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Mycobacterium leprae*

Inadvertent parenteral human-to-human transmission of leprosy has been reported following an accidental needle stick in a surgeon⁶¹ and the use of a presumably contaminated tattoo needle.⁶² There are no cases reported as a result of working in a

Agent Summary Statements – Bacterial Agents

laboratory with biopsy or other clinical materials of human or animal origin. While naturally occurring leprosy or leprosy-like diseases have been reported in armadillos⁶³ and in nonhuman primates,^{64,65} humans are the only known important reservoir of this disease.

Laboratory Hazards: 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.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious clinical materials from infected humans and animals. Extraordinary care should be taken to avoid accidental parenteral inoculation with contaminated sharp instruments. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for animal studies utilizing rodents, armadillos, and nonhuman primates. Vaccines are not currently available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Mycobacterium* spp. other than *M. tuberculosis*, *M. bovis* or *M. leprae*

Pike reported 40 cases of nonpulmonary "tuberculosis" thought to be related to accidents or incidents in the laboratory or autopsy room.⁶⁶ Presumably, these infections were due to mycobacteria other than *M. tuberculosis* or *M. bovis*. A number of mycobacteria that are ubiquitous in nature are associated with diseases other than tuberculosis or leprosy in humans, domestic animals, and wildlife. Characteristically, these organisms are infectious but not contagious. Clinically, the diseases associated with infections by these "atypical" mycobacteria can be divided into three general categories:

Agent Summary Statements – Bacterial Agents

1. **Pulmonary diseases resembling tuberculosis**, which may be associated with infection by *M. kansasii*, *M. avium* complex, and rarely, by *M. xenopi*, *M. malmoense*, *M. asiaticum*, *M. simiae*, and *M. szulgai*.
2. **Lymphadenitis**, which may be associated with infection by *M. scrofulaceum*, *M. avium* complex, and rarely, by *M. fortuitum* and *M. kansasii*.
3. **Skin ulcers and soft tissue wound infections**, which may be associated with infection by *M. ulcerans*, *M. marinum*, *M. fortuitum*, and *M. chelonae*.

Laboratory Hazards: The agents may be present in sputa, exudates from lesions, tissues, and in environmental samples (e.g., soil and water). 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. A potential infection hazard to laboratory personnel is also posed by the infectious aerosols created during the manipulation of broth cultures or tissue homogenates of these organisms associated with pulmonary disease.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacterium* spp. other than *M. tuberculosis* or *M. bovis*. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for animal studies with mycobacteria other than *M. tuberculosis*, *M. bovis*, or *M. leprae*. Vaccines are not currently available for use in humans.

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: *Mycobacterium tuberculosis*, *M. bovis*

Agent Summary Statements – Bacterial Agents

Mycobacterium tuberculosis and *M. bovis* (including BCG) infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory.^{67,68,69,70,71} 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.⁷² Naturally or experimentally infected nonhuman primates are a proven source of human infection (e.g., the annual tuberculin conversion rate in personnel working with infected nonhuman primates is about 70/10,000 compared with a rate of less than 3/10,000 in the general population).⁷³ Experimentally infected guinea pigs or mice do not pose the same problem since droplet nuclei are not produced by coughing in these species; however, litter from infected animals may become contaminated and serve as a source of infectious aerosols.

Laboratory Hazards: Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebrospinal fluid, urine, and in lesions from a variety of tissues.⁷⁴ Exposure to laboratory-generated aerosols is the most important hazard encountered. Tubercle bacilli may survive in heat-fixed smears,⁷⁵ 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* for humans (i.e., ID₅₀ <10 bacilli) and, in some laboratories, a high rate of isolation of acid-fast organisms from clinical specimens (>10%),⁷⁶ sputa, and other clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions.

Recommended Precautions: Biosafety Level 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 Class I or II biological safety cabinet. Use of a slide-warming tray, rather than flame-drying, is recommended. Liquification and concentration of sputa for acid-fast staining may also be conducted safely on the open bench by first treating the specimen (in a Class I or II safety cabinet) with an equal volume of 5% sodium hypochlorite solution

Agent Summary Statements – Bacterial Agents

(undiluted household bleach) and waiting 15 minutes before centrifugation.^{77,78,}

Biosafety Level 3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* or *M. bovis*, and for animal studies utilizing nonhuman primates experimentally or naturally infected with *M. tuberculosis* or *M. bovis*. Animal studies utilizing guinea pigs or mice can be conducted at Animal Biosafety Level 2.^{79,}

Note: Skin testing with purified protein derivative (PPD) of previously skin-tested-negative laboratory personnel can be used as a surveillance procedure. An attenuated live vaccine (BCG) is available but is not used in the United States for laboratory personnel. 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 (MMWR) for current vaccination recommendations.

Transfer of Agent: For a permit to import these agents, contact CDC. An importation or domestic transfer permit for *M. Bovis* can be obtained from USDA/APHIS/VS.

Agent: *Neisseria gonorrhoeae*

Laboratory-associated gonococcal infections have been reported in the United States.^{80,}

Laboratory Hazards: The agent may be present in conjunctival, urethral and cervical exudates, synovial fluid, urine, feces, and cerebrospinal fluid. Accidental parenteral inoculation and direct or indirect contact of mucous membranes with infectious clinical materials are the known primary laboratory hazards. The importance of aerosols is not determined.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical materials or

Agent Summary Statements – Bacterial Agents

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 Biosafety Level 3, may be indicated for aerosol or droplet production, and for activities involving production quantities or concentrations of infectious materials. Vaccines are not currently available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Neisseria meningitidis*

Meningococcal meningitis is a demonstrated but rare hazard to laboratory workers.^{81,82, 83.}

Laboratory Hazards: The agent may be present in pharyngeal exudates, cerebrospinal fluid, blood, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, infectious aerosol and ingestion are the primary hazards to laboratory personnel.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids, tissues, and cultures. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with a high potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials.

Note: Vaccines for *N. meningitidis* are available and should be considered for personnel regularly working with 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 (MMWR) for recommendations for vaccination against *N. meningitidis*.

Agent Summary Statements – Bacterial Agents

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Salmonella* - all serotypes except typhi

Salmonellosis is a documented hazard to laboratory personnel.^{84,85,86} 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.

Laboratory Hazards: 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.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially containing the agents. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals. Vaccines are not currently available for use in humans.

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: *Salmonella typhi*

Typhoid fever is a demonstrated hazard to laboratory personnel.^{87,88,89}

Laboratory Hazards: 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.

Agent Summary Statements – Bacterial Agents

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials and cultures. Biosafety Level 3 practices and procedures are recommended for activities likely to generate aerosols or for activities involving production quantities of organisms.

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 (MMWR) for recommendations for vaccination against *S. typhi*.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Shigella spp.*

Shigellosis is a demonstrated hazard to laboratory personnel, with dozens of cases reported in the United States and Great Britain alone.^{90,91,92,93} While outbreaks have occurred in captive nonhuman primates, humans are the only significant reservoir of infection. However, experimentally infected guinea pigs, other rodents, and nonhuman primates are also proven sources of infection.

Laboratory Hazards: The agent may be present in feces and, rarely, in the blood of infected humans or animals. Ingestion and parenteral inoculation of the agent are the primary laboratory hazards. The oral 25%-50% infectious dose of *S. flexneri* for humans is approximately 200 organisms.⁹⁴ The importance of aerosol exposure is not known.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Animal Biosafety Level 2 facilities and practices are recommended for activities with experimentally or naturally infect-

Agent Summary Statements – Bacterial Agents

ed animals. Vaccines are currently not available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC..

Agent: *Treponema pallidum*

Syphilis is a documented hazard to laboratory personnel who handle or collect clinical material from cutaneous lesions. Pike lists 20 cases of laboratory-associated infection.⁹⁵ Humans are the only known natural reservoir of the agent. Syphilis has been transmitted to laboratory personnel working with a concentrated suspension of *T. pallidum* obtained from an experimental rabbit orchitis.⁹⁶ Hematogenous transfer of syphilis has occurred from the transfusion of a unit of fresh blood obtained from a patient with secondary syphilis. *T. pallidum* is present in the circulation during primary and secondary syphilis. The minimum number (LD₅₀) of *T. pallidum* organisms needed to infect by subcutaneous injection is 23.⁹⁷ 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 strains of *T. pallidum* (Nichols and possibly others) retain their virulence for humans.

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

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or lesion materials from humans or infected rabbits. Gloves should be worn when there is a likelihood of direct skin contact with lesion materials. Periodic serological monitoring should be considered

Agent Summary Statements – Bacterial Agents

in personnel regularly working with infectious materials. Vaccines are not currently available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Vibrionic enteritis (Vibrio cholerae, V. parahaemolyticus)*

Vibrionic enteritis due to *Vibrio cholerae* or *Vibrio parahaemolyticus* is a documented but rare cause of laboratory-associated illness.⁹⁸ Naturally and experimentally infected animals are a potential source of infection.

Laboratory Hazards: Pathogenic vibrios may occur in feces. Ingestion of *V. cholerae* and ingestion or parenteral inoculation of other vibrios constitute the primary laboratory hazard. The human oral infecting dose of *V. cholerae* in healthy non-achlorhydric individuals is approximately 10^6 organisms.⁹⁹ The importance of aerosol exposure is not known. The risk of infection following oral exposure may be increased in achlorhydric individuals.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals. Although cholera vaccines exist, their routine use by laboratory staff has not been recommended. 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 (MMWR) for recommendations for vaccination against *V. cholerae*. There are currently no human vaccines against *V. parahaemolyticus*.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent Summary Statements – Bacterial Agents

Agent: *Yersinia pestis*

Plague is a proven but rare laboratory hazard; cases have been reported in the United States.^{100,101} Work with *Y. pestis* requires special security considerations due to its potential use for purposes of biological terrorism.

Laboratory Hazards: The agent may be present in bubo fluid, blood, sputum, cerebrospinal fluid (CSF), feces, and urine from humans, 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 rodents, infectious aerosols or droplets generated during the manipulation of cultures, and infected tissues. In the necropsy of rodents, primary hazards to laboratory personnel include accidental autoinoculation, ingestion, and bites by infected fleas collected from rodents.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the handling of potentially infectious clinical materials and cultures. Special care should be taken to avoid the generation of aerosols from infectious materials, and during the necropsy of naturally or experimentally infected rodents. Gloves should be worn when handling field-collected or infected laboratory rodents, and when there is the likelihood of direct skin contact with infectious materials. Necropsy of rodents is ideally conducted in a biological safety cabinet. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, are recommended for activities with high potential for droplet or aerosol production, for work with antibiotic-resistant strains, and for activities involving production quantities or concentrations of infectious materials.

Note: Vaccination for *Y. pestis* is available and should be considered for personnel working with infectious materials or infected rodents. The reader is advised to consult the current recommendations of the Advisory Committee on Immunization Practices (ACIP) published in the CDC Morbidity and Mortality

Agent Summary Statements – Bacterial Agents

Weekly Report (MMWR) for information on vaccination against *Y. pestis*.

Transfer of Agent: For a permit to import this agent, contact CDC. Contact the Department of Commerce for a permit to export this agent. Laboratory registration with CDC is required before sending or receiving this select agent.

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Section VII-B: Fungal Agents

Agent: *Blastomyces dermatitidis*

Laboratory-associated local infections have been reported following accidental parenteral inoculation with infected tissues or cultures containing yeast forms of *B. dermatitidis*.^{1,2,3,4,5,6,7,8} Pulmonary infections have occurred following the presumed inhalation of conidia; 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 (conidia).

Laboratory Hazards: Yeast forms may be present in the tissues of infected animals and in clinical specimens. Parenteral (subcutaneous) inoculation of these materials may cause local 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.

Recommended Precautions: Biosafety Level 2 and Animal Biosafety Level 2 practices and facilities are recommended for activities with clinical materials, animal tissues, cultures, environmental samples and infected animals.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Coccidioides immitis*

Laboratory-associated coccidioidomycosis is a documented hazard.^{11,12,13,14,15,16,17,18,19,20, 21,22} 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.²³

Laboratory Hazards: Because of the size (2-5 millimicrons), the arthroconidia are conducive to ready dispersal in air and retention in the deep pulmonary spaces. The much larger size of the spherule (30-60 millimicrons) considerably reduces the effectiveness of this form of the fungus as an airborne pathogen.

Agent Summary Statements – Fungal Agents

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. A theoretical laboratory hazard is posed by clinical specimens or tissues from infected animals or humans that have been stored or shipped in such a manner as to promote germination of arthroconidia. There is a single report of a veterinarian with coccidioidomycosis beginning 13 days after autopsy of a horse with that infection, though the veterinarian lived in an endemic area.²⁴ Accidental percutaneous inoculation of the spherule form may result in local granuloma formation.²⁵ Disseminated disease occurs at a much greater frequency in blacks and Filipinos than in whites.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues. Animal Biosafety Level 2 practices and facilities are recommended for experimental animal studies when the route of challenge is parenteral.

Biosafety Level 3 practices and facilities are recommended for propagating and manipulating sporulating cultures already identified as *C. immitis* and for processing soil or other environmental materials known or likely to contain infectious arthroconidia.

Transfer of Agent: For a permit to import this agent, contact CDC. Laboratory registration with CDC is required before sending or receiving this select agent.

Agent: *Cryptococcus neoformans*

Accidental inoculation of a heavy inoculum of *Cryptococcus neoformans* into the hands of laboratory workers has occurred during injection or necropsy of laboratory animals.^{26,27} Either a local granuloma or no lesion has resulted, suggesting low pathogenicity by this route. Respiratory infections as a consequence of laboratory exposure have not been recorded.

Agent Summary Statements – Fungal Agents

Laboratory Hazards: 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 droppings) may also represent a potential hazard to laboratory personnel.

Recommended Precautions: Biosafety Level 2 and Animal Biosafety Level 2 practices and facilities are recommended, respectively, for activities with known or potentially infectious clinical, environmental, or culture materials and with experimentally infected animals.

The processing of soil or other environmental materials known or likely to contain infectious yeast cells should be conducted in a Class I or Class II biological safety cabinet. This precaution is also indicated for culture of the perfect or sexual state of the agent.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Histoplasma capsulatum*

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.^{28,29,30} Pulmonary infections have resulted from handling mold form cultures.³¹ Local infection has resulted from skin puncture during autopsy of an infected human³² and from accidental needle inoculation of a viable culture.³³ Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers. Encapsulated spores 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. Furcolow reported that 10 spores were almost as effective as a lethal inoculum in mice as 10,000 to 100,000 spores.³⁴

Agent Summary Statements – Fungal Agents

Laboratory Hazards: 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.

Recommended Precautions: Biosafety Level 2 and Animal Biosafety Level 2 practices 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 experimental animal studies when the route of challenge is parenteral.

Biosafety Level 3 practices and facilities are recommended for propagating and manipulating cultures already identified as *H. capsulatum*, as well as processing soil or other environmental materials known or likely to contain infectious conidia.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: *Sporothrix schenckii*

S. schenckii has caused a substantial number of local skin or eye infections in laboratory personnel.³⁵ Most cases have been associated with accidents and have involved splashing culture material into the eye,^{36,37} scratching³⁸ or injecting³⁹ infected material into the skin or being bitten by an experimentally infected animal.^{40,41} Skin infections have resulted also from handling cultures^{42,43,44} or necropsy of animals⁴⁵ without a known break in technique. No pulmonary infections have been reported to result from laboratory exposure, although naturally occurring lung disease is thought to result from inhalation.

Recommended Precautions: Biosafety Level 2 and Animal Biosafety Level 2 practices and facilities are recommended for all laboratory and experimental animal activities with *S. schenckii*. Gloves should be worn when handling experimentally infected animals, and during operations with broth cultures that might result in hand contamination.

Agent Summary Statements – Fungal Agents

Transfer of Agent: For a permit to import this agent, contact CDC.

Agents: *Pathogenic Members of the Genera Epidermophyton, Microsporum, and Trichophyton*

Although skin, hair, and nail infections by these dermatophytid 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.^{46,47,48,49}

Laboratory Hazards: Agents are present in the skin, hair, and nails of human and animal hosts. Contact with infected laboratory animals with inapparent or apparent infections is the primary hazard to laboratory personnel. Cultures and clinical materials are not an important source of human infection.

Recommended Precautions: Biosafety Level 2 and Animal Biosafety Level 2 practices and facilities are recommended for all laboratory and experimental animal activities with dermatophytes. Experimentally infected animals should be handled with disposable gloves.

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: Miscellaneous Molds

Several molds have caused serious infection in immunocompetent hosts following presumed inhalation or accidental subcutaneous inoculation from environmental sources. These agents are *Penicillium marneffe*, *Exophiala (Wangiella) dermatitidis*, *Fonsecaea pedrosoi*, *Ochroconis gallopavum*, *Cladophialophora bantiana*, and *Ramichloridium mackenziei*, *Bipolaris* species. 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

Agent Summary Statements – Fungal Agents

precautions in the laboratory. *Penicillium marneffeii* has caused a local inoculation infection in a laboratory worker.⁵⁰ *Stachybotrys chartarum (atra)* is probably not a cause of infection or toxicosis in humans when the mold or fomites containing the mold are inhaled, although ingestion of moldy grain containing the fungus has poisoned animals.

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

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for propagating and manipulating cultures known to contain these agents.

Transfer of Agent: For a permit to import these agents, contact CDC.

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Agent Summary Statements – Fungal Agents

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Agent Summary Statements – Fungal Agents

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- **Section VII-C: Parasitic Agents**

Agent: Blood and Tissue Protozoal Parasites of Humans

Laboratory-associated infections with *Plasmodium* spp. (including *P. cynomolgi*); *Trypanosoma* spp.; and *Leishmania* spp. have been reported.^{1,2,3,4,5,6,7,8,9,10,11,12} Although no laboratory infections with *Babesia* spp. have been reported, they could result from accidental needlestick or from the bite of an infected tick.

Although laboratory animal-associated infections are not common, mosquito-transmitted malaria infections do occur. Other potential direct sources of infection for laboratory personnel include contact with lesion material from rodents with cutaneous leishmaniasis, and contact with feces or blood of animals or insects experimentally or naturally infected with *T. cruzi*.¹³

Laboratory Hazards: Infective stages may be present in blood, feces, cerebrospinal fluid (CSF), bone marrow, or other biopsy tissue, lesion exudates, and infected arthropods. Depending on the parasite, the primary laboratory hazards are ingestion, 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* spp., *T. cruzi*, or with tissue homogenates or blood containing hemoflagellates. Immunocompromised individuals should avoid working with live organisms.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for activities with infective stages of the parasites listed. Infected arthropods should be maintained in facilities which reasonably preclude the exposure of personnel or their escape to the outside. Primary containment (e.g., biological safety cabinet) or personal protection (e.g., face shield) may be indicated when working with cultures of *Leishmania* spp., *T. cruzi*, or with tissue homogenates or blood containing hemoflagellates.^{14,15} Gloves are recommended for activities where there is the likelihood of direct skin contact with infective stages of the parasites listed. Appropriate treatment for most protozoal

Agent Summary Statement – Parasitic Agents

infections exists, and information on dosage, source of drugs, etc., is available.¹⁶

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: Intestinal Protozoal Parasites of Humans

Laboratory-associated infections with *Toxoplasma* spp.; *Entamoeba* spp.; *Isospora* spp.; *Giardia* spp.; *Sarcocystis* spp.; and *Cryptosporidium* spp. have been reported.^{17,18,19,20,21} No laboratory infections with microsporidia have been reported, but they could result from ingestion of spores in feces, urine, sputum, CSF, or culture.

Laboratory animal-associated infections have been reported and provide a direct source of infection for laboratory personnel who come in contact with feces of experimentally or naturally infected animals. In the case of rodents experimentally-inoculated with *Toxoplasma* via the intraperitoneal route, contact with peritoneal fluid could result in exposure to infectious organisms.

Laboratory-related infections with *Cryptosporidium* have occurred with regularity in almost every laboratory working with this agent, especially those in which 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 may occur. Rigid adherence to protocol should reduce the occurrence in laboratory and animal care personnel.

Laboratory Hazards: Infective stages may be present in the feces or other body fluids and tissues. Depending on the parasite, ingestion is the primary laboratory hazard. Aerosol or droplet exposures of the mucous membranes of the eyes, nose, or mouth to trophozoites could pose potential hazards when working with cultures of free-living amoeba, such as *Naegleria fowleri*, *Acanthamoeba*, or *Balamuthia*, but the level of risk is unknown. Immunocompromised individuals should avoid working with live organisms. Because of the grave consequences of

Agent Summary Statement – Parasitic Agents

toxoplasmosis in the developing fetus, serologically negative women of childbearing age who might become pregnant should receive extensive counseling from a well-informed laboratory supervisor about the potential risks to the fetus. Fully informed employees who choose not to be exposed should be provided with alternative assignments in a work area where viable *Toxoplasma* organisms are not being handled. 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. Laboratories conducting studies only with killed or inactivated parasite materials, or parasite fractions, pose no significant risks.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for activities with infective stages of the parasites listed. Primary containment (e.g., biological safety cabinet) or personal protection (e.g., face shield) may be indicated when working with cultures of *Naegleria fowleri*, or *Cryptosporidium*. Appropriate treatment for most protozoal infections exists, and information on dosage, source of drugs, etc., is available.²²

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: Trematode Parasites of Humans (*Schistosoma* spp. and *Fasciola* spp.)

Laboratory-associated infections with *Schistosoma* spp. and *Fasciola* spp. have been reported. None have been directly associated with laboratory animals, with the exception of infected mollusk intermediate hosts.^{23,24,25,26}

Laboratory Hazards: Infective stages of *Schistosoma* spp. (cercariae) and *Fasciola* spp. (metacercaria) may be found, respectively, in the water or encysted on aquatic plants in laboratory aquaria used to maintain snail intermediate hosts. Skin penetration by schistosome cercariae and ingestion of fluke metacercaria

Agent Summary Statement – Parasitic Agents

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, metacercaria may be inadvertently transferred from hand to mouth by fingers or gloves, following contact with contaminated aquatic vegetation or surfaces of aquaria. Most laboratory exposures to *Schistosoma* spp. would predictably result in low worm burdens with minimal disease potential. Safe and effective drugs are available for the treatment of schistosomiasis.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for activities with infective stages of the parasites listed. Gloves should be worn when there may be direct contact with water containing cercariae, or vegetation containing metacercaria from naturally or experimentally infected snail intermediate hosts. Long-sleeved laboratory coats or other protective garb should be worn when working around aquaria or other water sources that may contain schistosome cercariae. Snails and cercariae in the water of laboratory aquaria should be killed by chemicals (e.g., hypochlorites, iodine) or heat before discharge to sewers. Appropriate treatment for most trematode infections exists, and information on source of drugs, dosage, etc. is available.²⁷

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: Cestode Parasites of Humans - *Echinococcus granulosus*, *Taenia solium* (*cysticercus cellulosae*) and *Hymenolepis nana*.

Although no laboratory-associated infections have been reported with either *E. granulosus* or *T. solium*, the consequences of such infections following the ingestion of infective eggs of *E. granulosus* or *T. solium* are potentially serious. *H. nana* is a cosmopolitan parasite which does not require an intermediate host and is directly transmissible by ingestion of feces of infected humans or rodents.

Agent Summary Statement – Parasitic Agents

Laboratory Hazards: Infective eggs may be present in the feces of dogs or other canids (the definitive hosts of *E. granulosus*), or in the feces of humans (the definitive host of *T. solium*). Ingestion of infective eggs from these sources is the primary laboratory hazard. Cysts and cyst fluids of *E. granulosus* are not infectious for humans. Ingestion of cysts containing the larval stage of *T. solium* (*Cysticercus cellulosae*) readily produces human infection with the adult tapeworm. With either parasite, 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 the definitive host could result in intestinal infection.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for work with infective stages of these parasites. Special attention should be given to personal hygiene practices (e.g., handwashing) and avoidance of ingestion of infective eggs. Gloves are recommended when there may be direct contact with feces or with surfaces contaminated with fresh feces of dogs infected with *E. granulosus*, with humans infected with *T. solium* adults, or with humans or rodents infected with *H. nana*. Appropriate treatment for many cestode infections exists, and information concerning source of drugs, dosage, etc., is available.²⁸

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: Nematode Parasites of Humans

Laboratory-associated infections with *Ascaris* spp.; *Strongyloides* spp.; hookworms; and *Enterobius* spp. have been reported.^{29,30,31} Allergic reactions to various antigenic components of nematodes (e.g., aerosolized *Ascaris* antigens) may represent an individual risk to sensitized persons. Laboratory animal-associated infections (including arthropods) have not been reported, but infective larvae in the feces of nonhuman primates infected with *Strongyloides* spp. are a potential infection hazard for laboratory and animal care personnel.

Agent Summary Statement – Parasitic Agents

Laboratory Hazards: Eggs and larvae in freshly passed feces of infected hosts are usually not infective; development to the infective stages may take periods of one day to several weeks. *Trichinella* is of concern since fresh or digested tissue may contain larvae and would be infective if ingested. Ingestion of the infective eggs or skin penetration of infective larvae are the primary hazards to laboratory and animal care personnel. Arthropods infected with filarial parasites pose a potential hazard to laboratory personnel. In laboratory personnel with frequent exposure to aerosolized antigens of *Ascaris* spp., development of hypersensitivity is common.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for activities with infective stages listed. Exposure to aerosolized sensitizing antigens of *Ascaris* spp. should be avoided. Primary containment (e.g., biological safety cabinet) may be required for work with these materials by hypersensitive individuals. Appropriate treatment for most nematode infections exists, and information on dosage, source of drugs, etc. is available.³²

Transfer of Agent: For a permit to import these agents, contact CDC.

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- **Section VII-D: Prions**

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Prions are **proteinaceous infectious** particles that lack nucleic acids.¹ Prions are composed largely, if not entirely, of an abnormal isoform of a normal cellular protein. In mammals, prions are composed of an abnormal, pathogenic isoform of the prion protein (PrP), designated PrP^{Sc}. The "Sc" superscript was initially derived from the term **scrapie** because scrapie is the prototypic prion disease. Since all of the known prion diseases (Table 6) of mammals involve aberrant metabolism of PrP similar to that observed in scrapie, use of the "Sc" superscript is suggested for all abnormal, pathogenic PrP isoforms.² In this context, the "Sc" superscript is used to designate the scrapie-like isoform of PrP.

A chromosomal gene encodes PrP and no PrP genes are found in purified preparations of prions. PrP^{Sc} is derived from PrP^C (the cellular isoform of PRP) by a posttranslational process whereby PrP^{Sc} acquires a high β -sheet content.³ Neither prion-specific nucleic acids nor virus-like particles have been detected in purified, infectious preparations. In fungi, evidence for three different prions has been accumulated.⁴

The mammalian prions cause scrapie and other related neurodegenerative diseases of humans and animals (Table 6). The prion diseases are also referred to as the transmissible spongiform encephalopathies (TSEs).⁵

Agent Summary Statement – Prions

Table 1. The Prion Diseases

<u>Disease abbreviation)</u>	<u>Natural Host</u>	<u>Prion</u>	<u>Pathogenic PrP Isoform</u>
Scrapie	sheep and goats	scrapie prion	OvPrP ^{Sc}
Transmissible mink encephalopathy (TME)	mink	TME prion	MkPrP ^{Sc}
Chronic wasting disease (CWD)	mule deer and elk	CWD prion	MdePrP ^{Sc}
Bovine spongiform encephalopathy (BSE)	cattle	BSE prion	BoPrP ^{Sc}
Feline spongiform encephalopathy (FSE)	cats	FSE prion	FePrP ^{Sc}
Exotic ungulate encephalopathy (EUE)	nyala and greater kudu	EUE prion	UngPrP ^{Sc}
Kuru	humans	kuru prion	HuPrP ^{Sc}
Creutzfeldt-Jakob disease (CJD)	humans	CJD prion	HuPrP ^{Sc}
Gerstmann-Sträussler-Scheinker syndrome (GSS)	humans	GSS prion	HuPrP ^{Sc}
Gatal familial insomnia (FFI)	humans	FFI prion	HuPrP ^{Sc}

Species-specificity of prions. Unlike many viruses, the properties of prions change dramatically when they are passaged from one species to another. The results of transgenic (Tg) mouse studies indicate that when human prions are passaged into mice, their potential non-Tg pathogenicity for humans probably declines drastically.⁶ The prions that are propagated in the non-Tg mice are now mouse prions, not human prions. The mouse prions contain mouse PrP^{Sc}, not human PrP^{Sc}. This

Agent Summary Statement – Prions

species-specific change in the PrP^{Sc} molecule is accompanied by an alteration in the pathogenicity of the prion. In contrast to the human prions, mouse prions are highly pathogenic for mice. Our understanding of these species-specific changes in prion pathogenicity is derived largely from studies of mice expressing a variety of PrP transgenes. Because the PrP^{Sc} produced in the mouse is from mouse PrP^C, it is not possible to determine the origin of the prion initially inoculated into the mouse.⁷

It is noteworthy that the susceptibility of a particular species to prions from another species can be profoundly affected by different prion strains.⁸ The properties manifested by prion strains such as incubation times and neuropathology profiles seem to be enciphered in the conformation of PrP^{Sc}.

Such considerations of the basic principles of prion biology help to form the basis for the biosafety classification of different prions.

Biosafety level classification. Human prions and those propagated in apes and monkeys are manipulated at Biosafety Level 2 or 3, depending on the studies being conducted. BSE prions are likewise manipulated at Biosafety Level 2 or 3, due to the possibility that BSE prions have been transmitted to humans in Great Britain and France.⁹

All other animal prions are considered Biosafety Level 2 pathogens. Thus, based on our current understanding of prion biology described above, once human prions are passaged in mice and mouse PrP^{Sc} is produced, these prions should be considered Biosafety Level 2 prions, even though the human prions are Biosafety Level 3 under most experimental conditions. An exception to this statement is in the case of mice expressing human or chimeric human/mouse transgenes. These transgenic mice produce human prions when infected with human prions and should be treated as Biosafety Level 2 or 3 in accord with the guidelines described above. The mechanism of prion spread among sheep and goats developing natural scrapie is unknown.^{10,11} CWD, TME, BSE, FSE, and EUE are all thought to occur after the consumption of prion-infected foods.^{12,13,14,15}

Agent Summary Statement – Prions

Human prion diseases. In the care of patients dying of human prion disease, the precautions used for patients with AIDS or hepatitis are certainly adequate. In contrast to these viral illnesses, the human prion diseases are not communicable or contagious.¹⁶ There is no evidence of contact or aerosol transmission of prions from one human to another. However, they are infectious under some circumstances, such as ritualistic cannibalism in New Guinea causing kuru, the administration of prion-contaminated growth hormone causing iatrogenic CJD, and the transplantation of prion-contaminated dura mater grafts.^{17,18,19} Familial CJD, GSS, and FFI are all dominantly inherited prion diseases; five different mutations of the PrP gene have been shown to be genetically linked to the development of inherited prion disease. Prions from many cases of inherited prion disease have been transmitted to apes, monkeys, and mice carrying human PrP transgenes.^{20,21,22}

Surgical procedures. Surgical procedures on patients diagnosed with prion disease should be minimized. It is thought that CJD has been spread from a CJD patient to two other patients who underwent neurosurgical procedures in the same operating theater shortly thereafter.²³ Although there is no documentation of the transmission of prions to humans through droplets of blood or cerebrospinal fluid, or by exposure to intact skin, or gastric and mucous membranes, the risk of such occurrences is a possibility. Sterilization of the instruments and decontamination of the operating room should be performed in accordance with recommendations described below.

Because it is important to establish a 'definitive' diagnosis of a human prion disease and to distinguish between sporadic and familial cases and those acquired by infection as a result of medical procedures or from prion-contaminated food products, unfixed brain tissue should be obtained. For all cases of suspected human prion disease, a minimum of one cubic centimeter of unfixed cerebral cortex should be part of any biopsy. This specimen should be bisected from the cortical surface through to the underlying white matter with one half of the specimen formalin-fixed and the other half frozen.

Agent Summary Statement – Prions

Autopsies. Routine autopsies and the processing of small amounts of formalin-fixed tissues containing human prions require Biosafety Level 2 precautions.²⁴ At autopsy, the entire brain should be collected and cut into coronal sections about 1.5 inches (~4 cm) thick; small blocks of tissue can easily be removed from each coronal section and placed in fixative for subsequent histopathologic analyses. Each coronal section is immediately heat sealed in a heavy-duty plastic bag. The outside of this bag is assumed to be contaminated with prions and other pathogens. With fresh gloves or with the help of an assistant with uncontaminated gloves, the bag containing the specimen is placed into another plastic bag which does not have a contaminated outer surface. The samples should then be frozen on dry ice or placed directly in a -70°C freezer for storage. At the very minimum, a coronal section of cerebral hemisphere containing the thalamus and one of the cerebellar hemisphere and brainstem should be taken and frozen.

The absence of any known effective treatment for prions disease demands caution. The highest concentrations of prions are in the central nervous system and its coverings. Based on animal studies, it is likely that high concentrations of prions are also found in spleen, thymus, lymph nodes, and lung. The main precaution to be taken when working with prion-infected or contaminated material is to avoid puncture of the skin.²⁵ The processor should wear cut-resistant gloves if possible. If accidental contamination of skin occurs, the area is swabbed with 1N sodium hydroxide for 5 minutes and then washed with copious amounts of water. Tables 2-5 provide guidelines to reduce the chance of skin punctures, aerosols, and contamination of operating room, morgue surfaces and instruments. Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care at Biosafety Level 3.

Bovine spongiform encephalopathy. The risk of infection for humans by BSE prions is unclear. Perhaps the most prudent approach is to study BSE prions in a Biosafety Level 2 or 3 facility depending on the samples to be studied, as noted above for human prions (i.e., brain, spinal cord).

Agent Summary Statement – Prions

Experimental rodent prion diseases. Mice and hamsters are the experimental animals of choice for all studies of prion disease. With the development of transgenic mice that are highly susceptible to human prions, the use of apes and monkeys is rarely needed. The highest titers of prions ($\sim 10^{9.5}$ ID₅₀/g) are found in the brain and spinal cord of laboratory rodents infected with adapted strains of prions,^{26,27} lower titers ($\sim 10^6$ ID₅₀/g) are present in the spleen and lymphoreticular system.^{28,29}

Physical properties of prions. The smallest infectious prion particle is probably a dimer of PrP^{Sc}; this estimate is consistent with an ionizing radiation target size of 55 ± 9 kDa.³⁰ Therefore, prions may not be retained by most of the filters that efficiently eliminate bacteria and viruses. Additionally, prions aggregate into particles of non-uniform size and cannot be solubilized by detergents, except under denaturing conditions where infectivity is lost.^{31,32} Prions resist inactivation by nucleases,³³ UV-irradiation at 254 nm,^{34,35} and treatment with psoralens,³⁶ divalent cations, metal ion chelators, acids (between pH 3 and 7), hydroxylamine, formalin, boiling, or proteases.^{37,38}

Inactivation of prions. Prions are characterized by extreme resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals (formalin, betapropiolactone, alcohols). While prion infectivity in purified samples is diminished by prolonged digestion with proteases,^{39,40} results from boiling in sodium dodecyl sulfate and urea are variable. Sterilization of rodent brain extracts with high titers of prions requires autoclaving at 132°C for 4.5 hours (h). Denaturing organic solvents such as phenol or chaotropic reagents such as guanidine isothiocyanate or alkali such as NaOH can also be used for sterilization.^{41,42,43,44,45} Prions are inactivated by 1N NaOH, 4.0 M guanidinium hydrochloride or isocyanate, sodium hypochlorite ($\geq 2\%$ free chlorine concentration), and steam autoclaving at 132°C for 4.5 h.^{46,47,48,49} It is recommended that dry waste be autoclaved at 132°C for 4.5 h or incinerated. Large volumes of infectious liquid waste containing high titers of prions can be completely sterilized by treatment with 1N NaOH (final concentration) or autoclaving at 132°C for 4.5 h. Disposable plasticware, which can be discarded

Agent Summary Statement – Prions

as a dry waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, the biosafety cabinets must be decontaminated with 1N NaOH, followed by 1N HCl, and rinsed with water. HEPA filters should be autoclaved and incinerated.

Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids. Formaldehyde-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. Some investigators recommend that formalin-fixed tissues from suspected cases of prion disease be immersed for 30 min in 96% formic acid or phenol before histopathologic processing,⁵⁰ but such treatment may severely distort the microscopic neuropathology.

Handling and processing of tissues from patients with suspected prion disease. The special characteristics of work with prions require particular attention to the facilities, equipment, policies, and procedures involved. The related considerations outlined in the following tables should be incorporated into the laboratory's risk management for this work.

Table 2. Standard precautions* for autopsies of patients with suspected prion disease

*Not to be confused with "Standard Universal Precautions"

1. Attendance should be limited to at least one experienced pathologist and minimal staff. One of the staff avoids direct contact with the deceased but assists with handling of instruments and specimen containers.
2. Standard autopsy attire is mandatory.
 - a. A disposable, waterproof gown is worn in place of a cloth gown.

Agent Summary Statement – Prions

- b. Cut-resistant gloves are worn underneath two pairs of surgical gloves or chain mail gloves are worn between two pairs of surgical gloves.
 - c. Aerosols are mainly created during opening of the skull with a Stryker saw. Appropriate respiratory protection should be worn (i.e., PAPR).
3. To reduce contamination of the autopsy suite:
- a. The autopsy table is covered with an absorbent sheet that has a waterproof backing.
 - b. Contaminated instruments are placed on an absorbent pad.
 - c. The brain is removed while the head is in a plastic bag to reduce aerosolization and splatter.
 - d. The brain can be placed into a container with a plastic bag liner for weighing.
 - e. The brain is placed onto a cutting board and appropriate samples are dissected for snap freezing (see Table 4).
 - f. The brain or organs to be fixed are immediately placed into a container with 10% neutral buffered formalin.
 - g. In most cases of suspected prion disease, the autopsy can be limited to examination of the brain only. In cases requiring a full autopsy, consideration should be given to examining and sampling of thoracic and abdominal organs *in situ*.

Table 3. Autopsy suite decontamination procedures

1. Instruments (open box locks and jaws) and saw blades are placed into a large stainless steel dish, soaked for 1 h in 2N sodium hydroxide or 2 h in 1N sodium hydroxide, and then rinsed well in water before autoclaving at 134°C (gravity displacement steam autoclaving for 1 h; porous load steam autoclaving for one 18-minute cycle at 30 lbs psi or six 3-minute cycles at 30 lbs psi).
2. The Stryker saw is cleaned by repeated wetting with 2N sodium hydroxide solution over a 1 h period. Appropriate washing to remove residual NaOH is required.

Agent Summary Statement – Prions

3. The absorbent table cover and instrument pads, disposable clothing, etc. are double bagged in appropriate infectious waste bags for incineration.
4. Any suspected areas of contamination of the autopsy table or room are decontaminated by repeated wetting over 1 h with 2N sodium hydroxide.

Table 4. Brain cutting procedures

1. After adequate formaldehyde fixation (at least 10-14 days), the brain is examined and cut on a table covered with an absorbent pad with an impermeable backing.
2. Samples for histology are placed in cassettes labeled with "CJD precautions." For laboratories that do not have embedding and staining equipment or microtome dedicated to infectious diseases including CJD, blocks of formalin-fixed tissue can be placed in 96% absolute formic acid for 30 minutes, followed by fresh 10% neutral buffered formalin solution for at least 48 h.⁵¹ The tissue block is then embedded in paraffin as usual. Standard neurohistological or immunohistochemical techniques are not obviously affected by formic acid treatment; however, in our experience, tissue sections are brittle and crack during sectioning.
3. All instruments and surfaces coming in contact with the tissue are decontaminated as described in Table 3.
4. Tissue remnants, cutting debris, and contaminated formaldehyde solution should be discarded within a plastic container as infectious hospital waste for eventual incineration.

Table 5. Tissue preparation

1. Histology technicians wear gloves, apron, laboratory coat, and face protection.

Agent Summary Statement – Prions

2. Adequate fixation of small tissue samples (e.g. biopsies) from a patient with suspected prion disease is followed by post-fixation in 96% absolute formic acid for 30 minutes, followed by 48 hours in fresh 10% formalin.
3. Liquid waste is collected in a 4L waste bottle containing 600 ml 6N sodium hydroxide.
4. Gloves, embedding molds, and all handling materials are disposed of as biohazardous waste.
5. Tissue cassettes are processed manually to prevent contamination of tissue processors.
6. Tissues are embedded in a disposable embedding mold. If used, forceps are decontaminated.
7. In preparing sections, gloves are worn, section waste is collected and disposed of in a biohazard waste receptacle. The knife stage is wiped with 1-2N NaOH, and the knife used is discarded immediately in a "biohazard sharps" receptacle. Slides are labeled with "CJD Precautions." The sectioned bloc is sealed with paraffin.
8. Routine staining:
 - a. Slides are processed by hand.
 - b. Reagents are prepared in 100 ml disposable specimen cups.
 - c. After placing the coverslip on, slides are decontaminated by soaking them for 1 h in 2N NaOH.
 - d. Slides are labeled as "Infectious-CJD."
9. Other suggestions:
 - a. Disposable specimen cups or slide mailers may be used for reagents.
 - b. Slides for immunocytochemistry may be processed in disposable petri dishes.
 - c. Equipment is decontaminated as described above.

Agent Summary Statement – Prions

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Agent Summary Statements: Rickettsial Agents

● Section VII-E: Rickettsial Agents

Agent: *Coxiella burnetii*

Of the rickettsial agents, *Coxiella burnetii* probably presents the greatest risk of laboratory infection. The organism is highly infectious and remarkably resistant to drying and environmental conditions.¹ The infectious dose of virulent Phase I organisms in laboratory animals has been calculated to be as small as a single organism. The estimated human ID₂₅₋₅₀ (inhalation) for Q fever is 10 organisms.² Pike's summary indicates that Q fever is the second most commonly reported laboratory-associated infection, with outbreaks involving 15 or more persons recorded in several institutions.^{3,4} A broad range of domestic and wild mammals are natural hosts for Q fever and 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.^{5,6} Although rare, *C. burnetii* is known to cause chronic infections such as endocarditis or granulomatous hepatitis.

Laboratory Hazards: The necessity of using embryonate eggs or cell culture techniques for the propagation of *C. burnetii* leads to extensive purification procedures. Exposure to infectious aerosols or parenteral inoculation are the most likely sources of infection to laboratory and animal care personnel.⁷ The agent may be present in infected arthropods, and in the blood, urine, feces, milk, and tissues of infected animal or human hosts. The placenta of infected sheep may contain as many as 10⁹ organisms per gram of tissue⁸ and milk may contain 10⁵ organisms per gram.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears. Biosafety Level 3 practices and facilities are recommended for activities involving the inoculation, incubation, and harvesting of embryonate eggs or cell cultures, the necropsy of infected animals and the manipulation of infected tissues.

Agent Summary Statements: Rickettsial Agents

Since infected guinea pigs and other rodents may shed the organisms in urine or feces,⁹ experimentally infected rodents should be maintained under Animal Biosafety Level 3. Specific clonal isolates of avirulent (Phase II) strains may be considered for lower containment conditions¹⁰.

Recommended precautions for facilities using sheep as experimental animals are described by Spinelli¹¹, and by Bernard.¹² An investigational new Phase I, Q fever vaccine (IND) is available from the Special Immunizations Program, U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), Fort Detrick, Maryland. The use of this vaccine should be limited to those who are at high risk of exposure and who have no demonstrated sensitivity to Q fever antigen. Individuals with valvular heart disease should not work with *C. burnetii*.

Transfer of Agent: For a permit to import this agent, contact CDC. Contact the Department of Commerce for a permit to export this agent. Laboratory registration with CDC is required before sending or receiving this select agent. An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Agent: *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*

Pike reported 57 cases of laboratory-associated typhus (type not specified), 56 cases of epidemic typhus with three deaths, and cases of murine typhus.¹³ More recently, three cases of murine typhus were reported from a research facility.¹⁴ Two of these three cases 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.

Agent Summary Statements: Rickettsial Agents

Rocky Mountain spotted fever is a documented hazard to laboratory personnel. Pike reported 63 laboratory-associated cases, 11 of which were fatal.¹⁵ Oster reported 9 cases occurring over a 6-year period in one laboratory, which were believed to have been acquired as a result of exposure to infectious aerosols.¹⁶

Laboratory Hazards: Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of laboratory-associated infection.¹⁷ Successful aerosol transmission of *R. rickettsii* has been experimentally documented in nonhuman primates.¹⁸ Five cases of rickettsial pox recorded by Pike were associated with exposure to bites of infected mites.¹⁹

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.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological and fluorescent antibody procedures, and for the staining of impression smears. Biosafety Level 3 practices 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 embryonate eggs or cell cultures. Animal Biosafety Level 2 practices and facilities are recommended for the holding of experimentally infected mammals other than arthropods. Level 3 practices and facilities are recommended for animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease.

Because of the proven value of antibiotic therapy in the early stages of infection, it is essential that laboratories working with rickettsiae have an effective system for reporting febrile illnesses in laboratory personnel, medical evaluation of potential cases and, when indicated, institution of appropriate antibiotic therapy. Vac-

Agent Summary Statements: Rickettsial Agents

cines are not currently available for use in humans (see following Surveillance section).

Transfer of Agent: For a permit to import these agents, contact CDC. Contact the Department of Commerce for a permit to export these agents. Laboratory registration with CDC is required before sending or receiving these select agents.

Surveillance of Personnel for Laboratory-Associated Rickettsial Infections

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. Delay in instituting appropriate chemotherapy, however, 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 surveillance system 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) a non-punitive atmosphere that encourages reporting of any febrile illness.

Rickettsial agents can be handled in the laboratory with minimal real danger to life when an adequate surveillance system complements a staff which is knowledgeable about the hazards of rickettsial infections and uses the safeguards recommended in the agent summary statements.

Agent Summary Statements: Rickettsial Agents

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Agent Summary Statements: Rickettsial Agents

- **Section VII-F: Viral Agents (other than arboviruses)**

Agent: Hantaviruses

Work with Hantaan virus (hemorrhagic fever with renal syndrome) and other hantaviruses (Puumala, Seoul, and Sin Nombre, whether or not they are registered in the *International Catalogue of Arboviruses and Certain Other Viruses-1985*, such as El Moro Canyon virus) in 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.

Hantavirus pulmonary syndrome (HPS) is a severe, often fatal new disease that is caused by Sin Nombre and/or related virus.¹ Most cases of human illness have resulted from exposures to naturally infected wild rodents. Arthropod vectors are not known to transmit hantaviruses. Person-to-person transmission has not been reported with any of the viruses associated with this disease.

Laboratory Hazards: Laboratory transmission of hantaviruses from rodents to humans via the aerosol route is well documented.^{2,3,4,5} Exposures to rodent excreta, 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.

Four laboratory workers were infected while working with cell-culture-adapted Hantaan virus. Although the procedures associated with infection are unclear, all four persons worked repeatedly with hantavirus cultures and performed centrifugation of concentrated virus.⁶ Viral RNA has been detected in necropsy specimens and in patient blood and plasma obtained early in the course of the disease.⁷ The implications of these findings for the infectivity of blood or tissues are unknown.

Recommended Precautions: Biosafety level 2 practices and procedures are recommended for laboratory handling of sera from persons potentially infected with the agents of HPS. The use of a certified biological safety cabinet is recommended for all

Agent Summary Statements – Viral Agents

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 should be carried out in a BSL-3 facility following BSL-3 practices and procedures. Large-scale growth of the virus, including preparing and handling viral concentrates, should be performed in BSL-4 containment facilities.

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

Transfer of Agents: For a permit to import these agents, contact CDC. Contact the Department of Commerce for a permit to export these agents. Laboratory registration with CDC is required before sending or receiving these select agents.

Agent: Hendra and Hendra-like Viruses (includes virus formerly known as Equine Morbillivirus)

Outbreaks of a previously unrecognized paramyxovirus, at first called equine morbillivirus, later named "Hendra virus," occurred in horses in Australia in 1994 and 1995.^{8,9,10,11,12} Three people in close contact with ill horses developed encephalitis or respiratory disease and two died. No associated outbreaks of human disease were recognized, but two piggery workers recalled an influenza-like illness at the time of the pig outbreak, and had neutralizing antibody titers to the Menangle virus. During 1998-99 an outbreak of illness caused by a similar but distinct Hendra-like virus occurred in Singapore and Malaysia.¹³ In Malaysia and

Agent Summary Statements – Viral Agents

Singapore, human illness, characterized by fever, severe headache, myalgia and signs of encephalitis has occurred in individuals in close contact with pigs (i.e., pig farmers and abattoir workers). Few patients developed a respiratory disease. Half of the people infected with this virus died. The natural host(s) for the Hendra and Hendra-like viruses has not been identified; however in Australia, bats are suspected of carrying the Hendra virus. Epidemiologic and laboratory studies are ongoing.

No laboratory-acquired infections are known to have occurred as a result of Hendra or Hendra-like virus exposure. However, it should be noted that in both the Australia and Malaysia/Singapore outbreaks the virus has been recognized as a significant veterinary pathogen. Laboratory studies have been confined to high containment veterinary and/or human infectious disease laboratories and veterinary and public health officials have monitored all studies closely.

Laboratory Hazards: The exact mode of transmission has not been established. All cases to date have been associated with close contact with horses, their blood or body fluids (Australia) or pigs (Malaysia/Singapore). Hendra and Hendra-like viruses have been isolated from tissues of infected animals in the outbreaks listed above. In the recent outbreak in Malaysia and Singapore, viral antigen has been found in central nervous system, kidney and lung tissues of fatal human cases.¹⁴

Recommended Precautions: Both 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 the Hendra, Hendra-like or suspected related viruses. Both human public health and veterinary disease experts should be involved in planning such laboratory studies, and the transport of specimens and isolates to the laboratory location. Until more information is available, handling of human clinical specimens or virus isolation attempts should be performed in, at least, enhanced BSL-3 facilities by experienced personnel. BSL-4 (suit laboratory or

Agent Summary Statements – Viral Agents

Class III safety cabinet) should be used for any work with infected animals or involving large quantities of virus.¹⁵

Transfer of Agent: For a permit to import this agent, contact CDC. An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS. Contact the Department of Commerce for a permit to export this agent. Laboratory registration with CDC is required before sending or receiving this select agent.

Agent: Hepatitis A Virus, Hepatitis E Virus

Laboratory-associated infections with hepatitis A or E viruses do not appear to be an important occupational risk among laboratory personnel. However, the disease is a documented hazard in animal handlers and others working with chimpanzees and other nonhuman primates which are naturally or experimentally infected.¹⁶ 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. Workers handling other recently captured, susceptible primates (owl monkeys, marmosets) may also be at risk.

Laboratory Hazards: The agents may be present in feces, saliva, and blood of infected humans and nonhuman primates. Ingestion of feces, stool suspensions, and other contaminated materials is the primary hazard to laboratory personnel. The importance of aerosol exposure has not been demonstrated. Attenuated or avirulent strains of hepatitis A viruses resulting from serial passage in cell culture been described.^{17,18}

Recommended Precautions: Biosafety Level 2 practices, safety equipment, and facilities are recommended for activities with known or potentially infected feces from humans or nonhuman primates. Animal Biosafety Level 2 practices and facilities are recommended for activities using naturally or experimentally infected nonhuman primates. Animal care personnel should wear gloves and take other appropriate precautions to avoid possible fecal-oral exposure. A licensed inactivated vaccine against hepatitis A is available in Europe; it is available as an investiga-

Agent Summary Statements – Viral Agents

tional vaccine in the U.S., and is recommended for laboratory personnel. Vaccines against hepatitis E are not available for use in humans.

Transfer of Agent: For a permit to import these agents, contact CDC.

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

Hepatitis B has been one of the most frequently occurring laboratory-associated infections,¹⁹ and laboratory workers are recognized as a high risk group for acquiring such infections.²⁰ Individuals who are infected with hepatitis B virus are at risk of infection with hepatitis D (delta) virus, which is defective and requires the presence of hepatitis B virus for replication.

Hepatitis C infection can occur in the laboratory situation. The prevalence of antibody to hepatitis C is slightly higher in medical care workers than in the general population. Epidemiologic evidence indicates that hepatitis C is spread predominantly by the parenteral route.^{21,22,23}

Laboratory Hazards: Hepatitis B virus may be present in blood and blood products of human origin, in urine, semen, cerebrospinal fluid, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin are the primary laboratory hazards. The virus may be stable in dried blood or blood components for several days. Attenuated or avirulent strains have not been identified.

Hepatitis C virus 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, repeated freezing and thawing, etc.

Recommended Precautions: Biosafety Level 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 precau-

Agent Summary Statements – Viral Agents

tions, such as those described for Biosafety Level 3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. Animal Biosafety Level 2 practices, containment equipment and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other nonhuman primates. Gloves should be worn when working with infected animals and when there is the likelihood of skin contact with infectious materials. Licensed recombinant vaccines against hepatitis B are available and are highly recommended for and offered to laboratory personnel.²⁴ Vaccines against hepatitis C and D are not yet available for use in humans.

In addition to these recommended precautions, persons working with HBV, HCV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.²⁵ Questions related to interpretation of this Standard should be directed to federal, regional or state OSHA offices.

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: *Herpesvirus simiae* (*Cercopithecine herpesvirus* [CHV-1], B-virus)

CHV-1 is a naturally occurring alphaherpesvirus infecting free-living or captive macaques including *Macaca mulatta*, *M. fascicularis*, and other members of the genus. In macaques it is associated with acute vesicular oral lesions, as well as latent and often recrudescence infection.²⁶ Human infection has been documented in at least 50 instances, usually with a lethal outcome or serious sequelae from encephalitis.^{27,28,29,30,31,32,33} Twenty-nine fatal cases of human infections (at a 58% fatality rate) with CHV-1 have been reported.^{34,35,36,37}

Although CHV-1 presents a potential hazard to laboratory personnel working with the agent, laboratory-associated human infections with CHV-1 have, with rare exceptions, been limited to those having direct contact with macaques. Primary macaque cell

Agent Summary Statements – Viral Agents

cultures, including commercially-prepared rhesus monkey kidney cells, occasionally may be asymptotically infected with CHV-1 and have been implicated in one human case.³⁸

Specific periodic training in risk assessment, understanding the modes of CHV-1 transmission and exposure, and proper use of personal protective equipment is highly recommended for all persons working with or having contact with macaques, their tissues, and their potentially-contaminated environments (including cages, enrichment toys, and waste materials). Appropriate immediate first-aid training and supplies and emergency medical support is necessary.

Laboratory Hazards: Asymptomatic shedding accounts for most transmission among monkeys and to human workers, although the highest risk of acquiring CHV-1 from macaques is through the bite of an infected monkey with active lesions. Contamination of broken skin or mucous membranes with oral, ocular, or urogenital secretions from infected macaques during their primary or recedescant infections is also dangerous and has caused at least one occupational fatality.³⁹ Stability of viral particles on cages and other surfaces is not known, but the potential hazard must be recognized for cuts or abrasions from these potentially-contaminated surfaces. Other alphaherpesviruses are not thought to persist in the environment for any duration. Experimental work with animals indicates that the importance of aerosol exposure of CHV-1 is likely to be minimal. Attenuated or avirulent strains have not been identified.

The agent also may be present in thoracic and abdominal viscera and nerve tissues of naturally infected macaques. These tissues, and the cultures prepared from them, are potential hazards.⁴⁰

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for all activities involving the use or manipulation of tissues, body fluids, and primary tissue culture

Agent Summary Statements – Viral Agents

materials from macaques. Additional practices and personnel precautions, such as those detailed for Biosafety Level 3, are recommended for activities involving the use or manipulation of any material known or suspected to contain CHV-1, including *in vitro* propagation of the virus for diagnosis. It would be prudent to confine manipulations of positive cultures which would contain high-titered virus to a BSL-4 facility (Class III BSC or suit laboratory; see Section III), depending on the judgement of the laboratory director.

Biosafety Level 4 practices and facilities are recommended for activities involving the propagation and manipulation of production quantities or concentrates of CHV-1.

All macaque colonies, even those thought to be free of CHV-1 antibody, should be presumed to be naturally infected. Animals with oral lesions suggestive of active B-virus infection should be identified and handled with extreme caution. Studies with animals experimentally infected with CHV-1 should be conducted at ABSL-3.

Guidelines are available for safely working with macaques and should be consulted.^{41,42} The wearing of gloves, masks, and laboratory coats or coveralls is recommended for all personnel while working with non-human primates – especially macaques and other Old World species – and for all persons entering animal rooms where non-human primates are housed. To minimize the potential for mucous membrane exposure,⁴³ some form of barrier must be utilized to prevent droplet splashes to eyes, mouth, and nasal passages. Types and use of personal protective equipment (e.g., goggles, glasses with solid side shields, or wrap-around face shields worn in conjunction with masks or respirators) must be determined with reference to the institutional hazard assessment. The specifications of the equipment must be balanced with the work to be performed so that the barriers selected do not increase work place risk by obscuring vision and contributing to increased risk of bites, needle sticks, or animal scratches.

Agent Summary Statements – Viral Agents

Antiviral drugs have shown promise in the therapy of rabbits infected with *H. simiae*, and limited clinical experience^{44,45} suggest that this may extend to man.^{46,47} Because of the seriousness of infection with this virus, experienced medical personnel should be available for consultation to manage incidents involving exposure to the agent or suspected infections. Human-to-human transmission has been documented in one case, indicating that precautions should be taken with vesicle fluids, oral secretions, and conjunctival secretions of infected persons.⁴⁸ Vaccines are not available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: Human Herpesviruses

The herpesviruses are ubiquitous human pathogens and are commonly present in a variety of clinical materials submitted for virus isolation. While few of these viruses are demonstrated causes of clinical laboratory-associated infections, they are primary as well as opportunistic pathogens, especially in immunocompromised hosts. Herpes simplex viruses 1 and 2 and varicella virus pose some risk via direct contact and/or aerosols; cytomegalovirus and Epstein-Barr virus pose relatively low infection risks to laboratory personnel. The risk of laboratory infection from herpesviruses 6 and 7 is not known. Although this diverse group of indigenous viral agents does not meet the criteria for inclusion in agent-specific summary statements (i.e., demonstrated or high potential hazard for laboratory-associated infection; grave consequences should infection occur), the frequency of their presence in clinical materials and their common use in research warrants their inclusion in this publication.

Laboratory Hazards: Clinical materials and isolates of herpesviruses may pose a risk of infection following ingestion, accidental parenteral inoculation, droplet exposure of the mucous membranes of the eyes, nose, or mouth, or inhalation of concentrated aerosolized materials. Clinical specimens containing

Agent Summary Statements – Viral Agents

the more virulent *Herpesvirus simiae* (B-virus) may be inadvertently submitted for diagnosis of suspected herpes simplex infection. This virus has also been found in cultures of primary rhesus monkey kidney cells. Cytomegalovirus may pose a special risk during pregnancy because of potential infection of the fetus.

Recommended Precautions: Biosafety Level 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 laboratory-associated infections, 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., biological safety cabinets) constitute the basic barrier protecting personnel from exposure to infectious aerosols.

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: Influenza

Laboratory-associated infections with influenza are not normally documented in the literature, but by informal accounts and published reports are known to have occurred, particularly when new strains showing antigenic drift or shift are introduced into a laboratory for diagnostic/research purposes.⁴⁹

Laboratory animal-associated infections are not reported; however, there is a high possibility of human infection from infected ferrets and vice-versa.

Laboratory Hazards: The agent may be present in respiratory tissues or secretions of humans or most infected animals, and in the cloaca of many infected avian species. The virus may be disseminated in multiple organs in some infected animal species.

Agent Summary Statements – Viral Agents

The primary laboratory hazard is inhalation of virus from aerosols generated by infected animals, or by aspirating, dispensing, or mixing virus-infected samples. Genetic manipulation has the potential for altering the host range, pathogenicity, and antigenic composition of influenza viruses. There is unknown potential for introducing into man transmissible viruses with novel antigenic composition.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended when receiving and inoculating routine laboratory diagnostic specimens. Autopsy material should be handled in a biological safety cabinet using Biosafety Level 2 procedures.

Activities Utilizing Noncontemporary Virus Strains: Biosafety considerations should take into account the available information about infectiousness and virulence of the strains being used, and the potential for harm to the individual or society in the event that laboratory-acquired infection and subsequent transmission occurs. Research or production activities utilizing contemporary strains may be safely performed using Biosafety Level 2 containment practices. Susceptibility to infection with older noncontemporary human strains, with recombinants, or with animal isolates warrant the use of Biosafety Level 2 containment procedures. However, there is no evidence for laboratory-acquired infection with reference strains A/PR/8/34 and A/W S/33, or its commonly used neurotropic variants.

Transfer of Agent: For a permit to import this agent, contact CDC. An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Agent: Lymphocytic Choriomeningitis Virus

Laboratory-associated infections with LCM virus are well documented in facilities where infections occur in laboratory rodents – especially mice, hamsters and guinea pigs.^{50,51,52} Nude and SCID mice may pose a special risk of harboring silent chronic infections. Cell cultures that have inadvertently become infected represent a potential source of infection and dissemination of the

Agent Summary Statements – Viral Agents

agent. Natural infections are found in nonhuman primates, including macaques and marmosets (*Callitrichid* hepatitis virus is a lymphocytic choriomeningitis virus) and may be fatal to marmoset monkeys. Swine and dogs are less important vectors.

Laboratory Hazards: The agent may be present in blood, cerebrospinal fluid, urine, secretions of the nasopharynx, feces and tissues of infected animal hosts and possibly man. 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.⁵³ The virus may pose a special risk during pregnancy because of potential infection of the fetus.

Recommended Precautions: Biosafety Level 2 practices and facilities are suitable for activities utilizing known or potentially infectious body fluids, and for cell culture passage of laboratory-adapted, mouse brain-passaged strains. Animal Biosafety Level 2 practices and facilities are suitable for studies in adult mice with mouse brain-passaged strains. However, additional primary containment and personnel precautions, such as those described for Biosafety Level 3, are indicated for activities with high potential for aerosol production, or involving production quantities or concentrations of infectious materials; and for manipulation of infected transplantable tumors, field isolates and clinical materials from human cases. Animal Biosafety Level 3 practices and facilities are recommended for work with infected hamsters. Vaccines are not available for use in humans.⁵⁴

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: Poliovirus

Laboratory-associated infections with polioviruses are uncommon and have been limited to unvaccinated laboratory personnel working directly with the agent.⁵⁵ There have been at least 12 documented laboratory associated poliovirus infections, including two deaths, between 1941 and 1976.⁵⁶ However, since only ~1% of infections with poliovirus result in disease, without laboratory

Agent Summary Statements – Viral Agents

confirmation it is impossible to estimate reliably the numbers of laboratory-acquired infections. With the available effective vaccines and vastly improved laboratory facilities, technologies and procedures, it is likely that such infections are now rare among laboratory workers. However, if laboratory workers do become infected, they provide a source of virus to exposed unvaccinated persons in the community.⁵⁷ Laboratory animal-associated infections have not been reported;⁵⁸ however, naturally or experimentally infected nonhuman primates could provide a source of infection to exposed unvaccinated persons. Transgenic mice expressing the human receptor for polioviruses can be experimentally infected by injection with virulent polioviruses and may be a potential source of human infection.

Laboratory Hazards: The agent is present in the feces and in throat secretions of infected persons. Ingestion or parenteral inoculation of infectious tissues or fluids by non-immunized personnel are the primary risks of infection in the laboratory. The importance of aerosol exposure is not known; it has not been reported as a hazard. Laboratory exposures pose negligible risk to appropriately immunized persons.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for all activities utilizing known or potentially infectious culture fluids and clinical materials involving known or suspected wild-type strains. All laboratory personnel working directly with the agent must have documented polio vaccination or demonstrated serologic evidence of immunity to all three poliovirus types.⁵⁹ Animal Biosafety Level 2 practices and facilities are recommended for studies of virulent viruses in animals. Unless there are strong scientific reasons for working with virulent polioviruses (which have been eradicated from the United States), laboratories should use the attenuated Sabin oral poliovirus vaccine strains. These pose no significant risk to immunized laboratory personnel.

The World Health Organization (WHO) has issued guidance documents⁶⁰ related to work with wild poliovirus in the near and long-term future. Starting in 1999, BSL-2/polio laboratories should be established for all workers wishing to manipulate wild

Agent Summary Statements – Viral Agents

poliovirus. BSL-2/polio follows traditional BSL-2 requirements for facilities, practices, and procedures, with the following additions: 1) all poliovirus stocks and potentially infectious materials are disposed of when there are no programmatic or research needs for retention; 2) all persons entering the laboratory are fully immunized against polio; 3) access to the laboratory is restricted; 4) all wild poliovirus retained in the laboratory is inventoried and stored in a separate secure area with limited access; 5) only viruses that are readily identifiable by molecular methods are used if wild virus reference strains or working stocks are required; and 6) Appropriate sterilization and/or incineration is used for disposing of wild polioviruses, infectious materials, and potentially infectious materials.

All laboratories wishing to retain wild poliovirus infectious or potentially infectious materials must begin implementing *BSL-3/polio* containment procedures one year after detection of the last wild poliovirus and provide documentation of implementation by the second year. Laboratories wishing to qualify as a BSL-3/polio facility and retain wild poliovirus infectious materials must then be listed on Agency/Institutional and National Inventories. Laboratories not wishing to convert to BSL-3/polio containment must destroy all wild poliovirus and potentially infectious materials by autoclaving or incineration. Alternatively, laboratories may contact a WHO-designated BSL-3/polio repository to arrange for transfer and storage of selected materials.

When OPV immunization stops, all work with wild poliovirus will be restricted to maximum containment (BSL-4) laboratories. These may be suit or cabinet laboratories (Section III).

Transfer of Agent: For a permit to import this agent, contact CDC.

Agent: Poxviruses

Sporadic cases of laboratory-associated infections with pox viruses (smallpox, vaccinia, yaba, tanapox) have been reported.⁶¹ Epidemiological evidence suggests that transmission of monkeypox virus to humans from nonhuman primates or rodents

Agent Summary Statements – Viral Agents

to humans may have occurred in nature, but not in the laboratory setting. Naturally or experimentally infected laboratory animals are a potential source of infection to exposed unvaccinated laboratory personnel. Genetically engineered recombinant vaccinia viruses pose an additional potential risk to laboratory personnel, through direct contact or contact with clinical materials from infected volunteers or animals.

Laboratory Hazards: The agents may be present in lesion fluids or crusts, respiratory secretions, or tissues of infected hosts. Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues, are the primary hazards to laboratory and animal care personnel. Some poxviruses are stable at ambient temperature when dried and may be transmitted by fomites.

Recommended Precautions: The possession and use of variola viruses is restricted to the World Health Organization Collaborating Center for Smallpox and Other Poxvirus Infections, located at the Centers for Disease Control and Prevention, Atlanta, Georgia. Biosafety Level 2 practices and facilities are recommended for all activities involving the use or manipulation of poxviruses, other than variola, that pose an infection hazard to humans. 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 documented evidence of satisfactory vaccination within the preceding ten years.^{62,63} Activities with vaccinia, cow pox, or monkey pox viruses, in quantities or concentrations greater than those present in diagnostic cultures, may also be conducted at Biosafety Level 2 by immunized personnel, provided that all manipulations of viable materials are conducted in Class I or II biological safety cabinets. Immunosuppressed individuals are at greater risk of severe disease if infected with a poxvirus.⁶⁴

Transfer of Agent: For a permit to import these agents, contact CDC. Contact the Department of Commerce for a permit to export these agents. Laboratory registration with CDC is required before sending or receiving these select agents.

Agent Summary Statements – Viral Agents

Agent: Rabies Virus

Laboratory-associated infections are extremely rare. Two have been documented. Both resulted from presumed exposure to high titered infectious aerosols, one generated in a vaccine production facility⁶⁵, and the other in a research facility.⁶⁶ Naturally or experimentally infected animals, their tissues, and their excretions are a potential source of exposure for laboratory and animal care personnel.

Laboratory Hazards: The agent may be present in all tissues of infected animals. Highest titers are present in CNS tissue, salivary glands, and saliva. Accidental parenteral inoculation, cuts, or 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 clinical materials and conducting diagnostic examinations. Fixed and attenuated strains of virus are presumed to be less hazardous, but the only two recorded cases of laboratory associated rabies resulted from exposure to a fixed Challenge Virus Standard (CVS) and an attenuated strain derived from SAD (Street Alabama Dufferin) strain, respectively.^{67,68}

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for all activities utilizing known or potentially infectious materials. Immunization is recommended for all individuals prior to working with rabies virus or infected animals, or engaging in diagnostic, production, or research activities with rabies virus. Immunization is also recommended for all individuals entering or working in the same room where rabies virus or infected animals are used. While it is not always feasible to open the skull or remove the brain of an infected animal within a biological safety cabinet, it is pertinent to wear heavy protective gloves to avoid cuts or sticks from cutting instruments or bone fragments, and to wear a face shield to protect the mucous membranes of the eyes, nose, and mouth from exposure to infectious droplets or tissue fragments. If a Stryker saw is used to open the skull, avoid contacting the brain with the blade of the

Agent Summary Statements – Viral Agents

saw. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with a high potential for droplet or aerosol production, and for activities involving production quantities or concentrations of infectious materials.

Transfer of Agent: For a permit to import this agent, contact CDC.

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

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.⁶⁹

In 1992, two workers in different laboratories were reported to have developed antibodies to simian immunodeficiency virus (SIV) following exposures. One was associated with a needle stick which occurred while the worker was manipulating a blood-contaminated needle after bleeding an SIV-infected macaque monkey.⁷⁰ The other 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.⁷¹ The first worker seroconverted and has no evidence of persistent SIV infection. The second worker has been

Agent Summary Statements – Viral Agents

seropositive for at least nine years with no evidence of illness or immunological incompetence.

Recent publications^{72,73} have identified the prevalence (4/231, 1.8%) of infection with simian foamy viruses (SFV) among humans occupationally exposed to nonhuman primates. Evidence of SFV infections included seropositivity, proviral DNA detection, and isolation of foamy virus. The infecting SFV originated from an African green monkey (one person) and baboons (three people). These infections have not as yet resulted in either disease or sexual transmission, and may represent benign endpoint infections.

Laboratory Hazards: HIV has been isolated from blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, cervical secretion, and tissue of infected persons and experimentally infected nonhuman primates.⁷⁴ CDC has recommended that blood and body fluid precautions be used consistently when handling any blood-contaminated specimens.^{75,76} This approach, referred to as "universal precautions," precludes the need to identify clinical specimens obtained from HIV-positive patients or to speculate as to the HIV status of a specimen.

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, cerebrospinal fluid, and a variety of tissues of infected nonhuman primates. Limited data exist on the concentration of virus in semen, saliva, cervical

Agent Summary Statements – Viral Agents

secretions, urine, breast milk, and amniotic fluid. In the laboratory, virus should be presumed to be present in all SIV cultures, in animals experimentally infected or inoculated with SIV, in all materials derived from HIV or SIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.⁷⁷

In the laboratory, 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. 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 virus-containing or potentially infected materials.⁷⁸

Recommended Precautions:

In addition to the following recommended precautions, persons working with HIV, SIV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.⁷⁹ Questions related to interpretation of this Standard should be directed to Federal, regional or state OSHA offices.

1. BSL-2 standard and special practices, containment equipment and facilities are recommended for activities involving **all** blood-contaminated clinical specimens, body fluids and tissues from **all** humans, or from HIV- or SIV-infected or inoculated laboratory animals.
2. 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, but using the additional practices and containment equipment recommended for BSL-3.

Agent Summary Statements – Viral Agents

3. Activities involving industrial-scale volumes or preparation of concentrated HIV or SIV are conducted in a BSL-3 facility, using BSL-3 practices and containment equipment.
4. Nonhuman primates or other animals infected with HIV or SIV are housed in ABSL-2 facilities using ABSL-2 special practices and containment equipment.

Additional Comments:

1. There is no evidence that laboratory clothing poses a risk for retrovirus transmission; however, clothing that becomes contaminated with HIV or SIV should be decontaminated before being laundered or discarded. Laboratory personnel must remove laboratory clothing before going to non-laboratory areas.
2. Work surfaces are decontaminated with an appropriate chemical germicide after procedures are completed, when surfaces are overtly contaminated, and at the end of each work day. Many commercially available chemical disinfectants^{80,81,82,83,84}, can be used for decontaminating laboratory work surfaces and some laboratory instruments, for spot cleaning of contaminated laboratory clothing, and for spills of infectious materials. Prompt decontamination of spills should be standard practice.
3. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2.
4. It is recommended that all institutions establish written policies regarding the management of laboratory exposure to HIV and SIV in conjunction with applicable federal, state and local laws. Such policies should consider confidentiality, consent for testing, administration of appropriate prophylactic drug therapy,⁸⁵ counseling, and other related issues. If a laboratory worker has a parenteral or mucous-membrane exposure to blood, body fluid, or viral-culture material, the source material should be identified and, if possible, tested for the presence of virus. If the source material is positive for

Agent Summary Statements – Viral Agents

HIV antibody, virus, or antigen, or is not available for examination, the worker should be counseled regarding the risk of infection and should be evaluated clinically and serologically for evidence of HIV infection. Post-exposure prophylaxis should be offered according to the latest guidelines. The worker should be advised to report and seek medical evaluation of any acute febrile illness that occurs within 12 weeks after the exposure.⁸⁶ Such an illness – particularly one characterized by fever, rash, or lymphadenopathy – may indicate recent HIV infection. If the initial (at time of exposure) test is negative, the worker should be retested 6 weeks after the exposure and periodically thereafter (i.e., at 12 weeks and 6, 9 and 12 months after exposure). During this follow-up period exposed workers should be counseled to follow Public Health Service recommendations for preventing transmission of HIV.^{87,88,89,90,91}

5. Other primary and opportunistic pathogenic agents may be present in the body fluids and tissues of persons infected with HIV. Laboratory workers should follow accepted biosafety practices to ensure maximum protection against inadvertent laboratory exposure to agents that may also be present in clinical specimens or in specimens obtained from nonhuman primates.^{92,93,94}

Research involving other human (i.e., human T-lymphotropic virus types I and II) and simian retroviruses occurs in many laboratories. Recently, surveillance for such infections revealed occupational exposure and infection by simian foamy virus among animal caretakers at laboratory research facilities.^{95,96} The precautions outlined above are sufficient while working with these agents.

Laboratory work with retroviral vectors, especially those containing full-length infectious molecular genomes (HIV-1), should be handled in BSL-2 facilities under BSL-2/3 practice. This includes infectious clones derived from nonhuman viruses, but possessing xenotropic (especially for human cells) host ranges.

Agent Summary Statements – Viral Agents

Transfer of Agent: For a permit to import these agents, contact CDC.

Agent: *Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru and related agents)*

Laboratory-associated infections with the transmissible spongiform encephalopathies (prion diseases) have not been documented. However, there is evidence that Creutzfeldt-Jakob disease (CJD) has been transmitted iatrogenically to patients by corneal transplants, dura mater grafts and growth hormone extracted from human pituitary glands, and by exposure to contaminated electroencephalographic electrodes.⁹⁷ Infection is always fatal. There is no known nonhuman reservoir for CJD or kuru. Nonhuman primates and other laboratory animals have been infected by inoculation, but there is no evidence of secondary transmission. Scrapie of sheep and goats, bovine spongiform encephalopathy and mink encephalopathy are transmissible spongiform encephalopathies of animals that are similar to the human transmissible diseases. However, there is no evidence that the animal diseases can be transmitted to man. (See also Section VII-D, Prions.)

Laboratory Hazards: High titers of a transmissible agent have been demonstrated in the brain and spinal cord of persons with kuru. In persons with Creutzfeldt-Jakob disease and its Gerstmann-Sträussler-Schenker Syndrome variants, a similar transmissible agent has been demonstrated in the brain, spleen, liver, lymph nodes, lungs, spinal cord, kidneys, cornea and lens, and in spinal fluid and blood. Accidental parenteral inoculation, especially of nerve tissues, including formalin-fixed specimens, is extremely hazardous. Although non-nerve tissues are less often infectious, all tissues of humans and animals infected with these agents should be considered potentially hazardous. The risk of infection from aerosols, droplets, and exposure to intact skin, gastric and mucous membranes is not known; however, there is no evidence of contact or aerosol transmission. These agents are characterized by extreme resistance to conventional inactivation procedures including irradiation, boiling, dry heat and chemicals (formalin, betapropiolactone, alcohols); however, they are

Agent Summary Statements – Viral Agents

inactivated by 1 N NaOH, sodium hypochlorite ($\geq 2\%$ free chlorine concentration) and steam autoclaving at 132°C for 4.5 hours.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended for all activities utilizing known or potentially infectious tissues and fluids from naturally infected humans and from experimentally infected animals. Extreme care must be taken to avoid accidental autoinoculation or other traumatic parenteral inoculations of infectious tissues and fluids.⁹⁸ Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids, and during the necropsy of experimental animals. It is further strongly recommended that gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids. Formaldehyde-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. It is recommended that formalin-fixed tissues from suspected cases of transmissible encephalopathy be immersed in 96% formic acid for 30 minutes before histopathologic processing.⁹⁹ Vaccines are not available for use in humans.¹⁰⁰

Transfer of Agent: For a permit to import these agents, contact CDC. An importation or domestic transfer permit for Bovine spongiform encephalopathy can be obtained from USDA/APHIS/VS.

Agent: Vesicular Stomatitis Virus

A number of laboratory-associated infections with indigenous strains of VSV have been reported.¹⁰¹ Laboratory activities with such strains present two different levels of risk to laboratory personnel and are related, at least in part, to the passage history of the strains utilized. Activities utilizing infected livestock, their infected tissues, and virulent isolates from these sources are a demonstrated hazard to laboratory and animal care personnel.^{102,103} Rates of seroconversion and clinical illness in personnel working with these materials are high.¹⁰⁴ Similar risks may be associated with exotic strains such as Piriy.¹⁰⁵

Agent Summary Statements – Viral Agents

In contrast, anecdotal information indicates that activities with less virulent laboratory-adapted strains (e.g., Indiana, San Juan and Glasgow) are rarely associated with seroconversion or illness. Such strains are commonly used by molecular biologists, often in large volumes and high concentrations, under conditions of minimal or no primary containment. Some strains of VSV are considered restricted organisms by USDA regulations (9CFR 122.2). Experimentally infected mice have not been a documented source of human infection.

Laboratory Hazards: The agent may be present in vesicular fluid, tissues, and blood of infected animals and in blood and throat secretions of infected humans. Exposure to infectious aerosols, infected droplets, direct skin and mucous membrane contact with infectious tissues and fluids, and accidental autoinoculation, are the primary laboratory hazards associated with virulent isolates. Accidental parenteral inoculation and exposure to infectious aerosols represent potential risks to personnel working with less virulent laboratory-adapted strains.

Recommended Precautions: Biosafety Level 3 practices and facilities are recommended for activities involving the use or manipulation of infected tissues and virulent isolates from naturally or experimentally infected livestock. Gloves and respiratory protection are recommended for the necropsy and handling of infected animals. Biosafety Level 2 practices and facilities are recommended for activities utilizing laboratory-adapted strains of demonstrated low virulence. Vaccines are not available for use in humans.

Transfer of Agent: For a permit to import this agent, contact CDC. A USDA importation or domestic transfer permit for this agent is also required and can be obtained from USDA/APHIS/VS.

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- **Section VII-G: Arboviruses and Related Zoonotic Viruses**

Arboviruses Assigned to Biosafety Level 2

The American Committee on Arthropod-Borne Viruses (ACAV) registered 537 arboviruses as of December 1997. In 1979, the ACAV's Subcommittee on Arbovirus Laboratory Safety (SALS) categorized each of the 424 viruses then registered in the *Catalogue of Arboviruses and Certain Other Viruses of Vertebrates*¹ into one of four groups of recommended practices, safety equipment, and facilities. These are described in this publication as Biosafety Levels 1-4.² SALS has periodically updated the 1980 publication by providing a supplemental listing and recommended levels of practice and containment for arboviruses registered since 1979.

SALS categorizations are based on risk assessments derived from information provided by a worldwide survey of 585 laboratories working with arboviruses. SALS recommends that work with the majority of these agents be conducted at the equivalent of Biosafety Level 2 (Table 1). SALS also recognizes five commonly used vaccine strains for which attenuation is firmly established. These viruses may be handled safely at BSL-2 provided that personnel working with these vaccine strains are immunized (Table 2). SALS has classified all registered viruses for which insufficient laboratory experience exists as BSL-3 (Table 3), and reevaluates the classification whenever additional experience is reported.

The viruses classified as BSL-2 are listed alphabetically in Table 1 on pages 186 through 188 and include the following agents that are reported to have caused laboratory-associated infections.^{3,4,5}

Virus	Cases
Vesicular stomatitis**	46
Colorado tick fever	16
Dengue*	11
Pichinde	17

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

Virus	Cases
Western equine encephalomyelitis**	7 (2 deaths)
Rio Bravo	7
Kunjin	6
Catu	5
Caraparu	5
Ross River	5
Bunyamwera	4
Eastern equine encephalomyelitis* - **	4
Zika	4
Apeu	2
Marituba	2
Tacaribe	2
Murutucu	1
O'nyong nyong	1
Modoc	1
Oriboca	1
Ossa	1
Keystone	1
Bebaru	1
Bluetongue* - **	1

* Export license required by Department of Commerce

** An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

The results of the SALS survey clearly indicate that the suspected source of the laboratory-associated infections listed above was other than exposure to infectious aerosols. The recommendation that work with the 342 arboviruses listed in Table 1 be conducted at Biosafety Level 2 was based on the existence of adequate historical laboratory experience to assess the risks when working with this group of viruses risks. This indicates that either (a) no overt laboratory-associated infections are reported; (b) infections resulted from exposures other than to infectious aerosols; or if disease from aerosol exposure is documented, it is uncommon.

Laboratory Hazards: Agents listed in this group may be present in blood, CSF, central nervous system and other tissues,

Agent Summary Statements—Arboviruses/Related Zoonotic Viruses

and infected arthropods, depending on the agent and the stage of infection. The primary laboratory hazards are posed by accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites of infected laboratory rodents or arthropods. However, infectious aerosols may also be a potential source of infection.

Recommended Precautions: Biosafety Level 2 practices, safety equipment, and facilities are recommended for activities with potential infectious clinical materials and arthropods and for manipulations of infected tissue cultures, embryonate eggs, and rodents. Infection of newly hatched chickens with eastern and western equine encephalomyelitis viruses is especially hazardous and should be undertaken only by immunized personnel under Biosafety Level 3 conditions. Investigational vaccines (IND) against eastern equine encephalomyelitis and western equine encephalomyelitis viruses are available through the Centers for Disease Control and Prevention (telephone 404-639-3356) and the U.S. Army Medical Research Institute for Infectious Diseases, (USAMRIID) Fort Detrick, Maryland (telephone 301-619-2833). The use of these vaccines is recommended for personnel who work directly and regularly with these two agents in the laboratory.

Prior to 1988, 12 laboratory-acquired dengue infections were reported. However, from 1988 through 1991, four additional cases have been documented. In all four cases, proper protective gear (long-sleeved lab gowns tying in back, gloves, masks, safety glasses) was not worn, and in three instances, containment of potential aerosols in a laminar flow biosafety cabinet was ignored. These aerosols or infected fluids most likely contaminated broken, unprotected skin. An additional factor in these cases was work with highly concentrated amounts of virus. Safe manipulation of dengue viruses in the laboratory (particularly in concentrated preparations) requires strict adherence to Biosafety Level 2 recommendations.

Large quantities and/or high concentrations of any virus have the potential to overwhelm both natural immune mechanisms and vaccine-induced immunity. When a virus from Biosafety Level 2

Agent Summary Statements—Arboviruses/Related Zoonotic Viruses

is being produced in large quantities or in high concentrations, laboratory directors should ensure that proper protective gear is utilized, as described in the above paragraph, and that manipulations are performed in laminar flow biosafety cabinets. (See also Section V, Risk Assessment.)

Transfer of Agents: For a permit to import this agent, contact CDC. Laboratory registration with CDC may be required before sending or receiving some of these select agents.

Table 1. Arboviruses and Arenaviruses Assigned to Biosafety Level 2

Acado	Banzi	Calovo
Acara	Barmah Forest	Candiru
Aguacate	Barur	Cape Wrath
Alfuy	Batai	Capim
Almpiwar	Batama	Caraparu
Amapari	Bauline	Carey Island
Ananindeua	Bebaru	Catu
Anhanga	Belmont	Chaco
Anhembi	Benevides	Chagres
Anopheles A	Benfica	Chandipura
Anopheles B	Bertioga	Changuinola
Apeu	Bimiti	Charleville
Apoi	Birao	Chenuda
Aride	Bluetongue*	Chilibre
Arkonam	Boraceia	Chobar gorge
Aroa	Botambi	Clo Mor
Aruac	Boteke	Colorado tick fever
Arumowot	Bouboui	Corriparta
Aura	Bujaru	Cotia
Avalon	Bunyamwera	Cowbone Ridge
Abras	Bunyip Creek	Csiro Village
Abu Hammad	Burg El Arab	Cuiaba
Babahoyo	Bushbush	D'Aguilar
Bagaza	Bussuquara	Dakar Bat
Bahig	Buttonwillow	Dengue-1
Bakau	Bwamba	Dengue-2
Baku	Cacao	Dengue-3
Bandia	Cache Valley	Dengue-4
Bangoran	Caimito	Dera Ghazi Khan
Bangui	California enc.	East. equine

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

enceph.** ◇	Jutiapa	Madrid
Edge Hill	Kadam	Maguari
Entebbe Bat	Kaeng Khoi	Mahogany hammock
Ep. Hem. Disease	Kaikalur	Main Drain
Erve	Kaisodi	Malakal
Eubenangee	Kamese	Manawa
Eyach	Kammavanpettai	Manitoba
Flanders	Kannamangalam	Manzanilla
Fort Morgan	Kao Shuan	Mapputta
Frijoles	Karimabad	Maprik
Gamboia	Karshi	Marco
Gan Gan	Kasba	Marituba
Gomoka	Kemerovo	Marrakai
Gossas	Kern Canyon	Matariya
Grand Arbaud	Ketapang	Matruh
Great Island	Keterah	Matucare
Guajara	Keuraliba	Melao
Guama	Keystone	Mermet
Guaratuba	Kismayo	Minatitlan
Guaroa	Klamath	Minnal
Gumbo Limbo	Kokobera	Mirim
Hart Park	Kolongo	Mitchell River
Hazara	Koongol	Modoc
Highlands J	Kotonkan	Moju
Huacho	Kowanyama	Mono Lake
Hughes	Kunjin	Mont. myotis leuk
Icoaraci	Kununurra	Moriche
Ieri	Kwatta	Mosqueiro
Ilesha	La Crosse	Mossuril
Ilheus	La Joya	Mount Elgon bat
Ingwavuma	Lagos Bat	Murutucu
Inkoo	Landjia	Mykines
Ippy	Langat	Navarro
Irituia	Lanjan	Nepuyo
Isfahan	Las Maloyas	Ngaingan
Itaporanga	Latino	Nique
Itaqui	Le Dantec	Nkolbisson
Jamestown Canyon	Lebombo	Nola
Japanaut	Lednice	Ntaya
Johnson Atoll	Lipovnik	Nugget
Joinjakaka	Lokern	Nyamanini
Juan Diaz	Lone Star	Nyando
Jugra	Lukuni	O'nyong-nyong
Jurona	M'poko	Okhotskiy

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

Okola	Sango	Toscana
Olifantsvlei	Sathuperi	Toure
Oriboca	Sawgrass	Tribec
Ossa	Sebokele	Triniti
Pacora	Seletar	Trivittatus
Pacui	Sembalam	Trubanaman
Pahayokee	Serra do Navio	Tsuruse
Palyam	Shamonda	Turlock
Parana	Shark River	Tyulenyi
Pata	Shuni	Uganda S
Pathum Thani	Silverwater	Umatilla
Patois	Simbu	Umbre
Phnom-Penh bat	Simian hem. fever	Una
Pichinde	Sindbis	Upolu
Pixuna	Sixgun City	Urucuri
POngola	Snowshoe hare	Usutu
Ponteves	Sokuluk	Uukuniemi
Precairous Point	Soldado	Vellore
Pretoria	Sororoca	Venkatapuram
Prospect Hill	Stratford	Vinces
Puchong	Sunday Canyon	Virgin River
Punta Salinas	Tacaiuma	VS-Indiana
Punta Toro	Tacaribe	VS-New Jersey
Qalyub	Taggert	Wad Medani
Quaranfil	Tahyna	Wallal
Restan	Tamiami	Wanowrie
Rio Bravo	Tanga	Warrego
Rio Grande	Tanjong Rabok	West. equine enc.** ◇
Ross River	Tataguine	Whataroa
Royal Farm	Tehran	Witwatersrand
Sabo	Tembe	Wongal
Saboya	Tembusu	Wongorr
Saint Floris	Tensaw	Wyeomyia
Sakhalin	Tete	Yaquina Head
Salehabad	Tett nang	Yata
San Angelo	Thimiri	Yogue
Sandfly fever (Naples)	Thottapalayam	Zaliv Terpeniya
Sandfly fever (Sicilian)	Tibrogargan	Zegla
Sandjimba	Timbo	Zika
	Timboteua	Zirqa
	Tindholmur	

* Export permit required by Department of Commerce.

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

- ** A vaccine is available and is recommended for all persons working with this agent.
- ◇ An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Transfer of Agent: For a permit to import these agents, contact CDC. Laboratory registration with CDC is required before sending or receiving these select agents.

Table 2. Vaccine Strains of BSL-3/4 Viruses Which May Be Handled at Biosafety Level-2

<u>Virus</u>	<u>Vaccine Strain</u>
Chikungunya*	131/25
Junin*	Candid #1
Rift Valley fever*	20MP-12
Venezuelan equine encephalomyelitis*	TC-83
Yellow fever*	17-D

* Export permit may be required by Department of Commerce

Arboviruses and Arenaviruses Assigned to Biosafety Level 3

SALS recommends that work with the 184 arboviruses included in the alphabetical listings of Tables 3 and 4 be conducted at the equivalent of Biosafety Level 3 practices, safety equipment, and facilities. These recommendations are based on the following criteria: for Table 3 (pages 193-194), SALS considered the laboratory experience inadequate to assess risk, regardless of the available information regarding disease severity. For the agents listed on Table 4 (pages 194-195), SALS recorded overt laboratory-associated infections 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. Arboviruses were also classified BSL-3 if they cause diseases in domestic animals in countries outside the USA.

Agent Summary Statements—Arboviruses/Related Zoonotic Viruses

Laboratory or laboratory animal-associated infections have been reported with the following BSL-3 agents:^{6,7,8.}

Virus	Cases (SALS)
Venezuelan equine encephalomyelitis* ◇	150 (1 death)
Rift Valley fever* ◇	47 (1 death)
Chikungunya*	39
Yellow fever*	38 (8 deaths)
Japanese encephalitis*	22
Louping ill ◇	22
West Nile	18
Lymphocytic choriomeningitis*	15
Orungo	13
Piry ◇	13
Wesselsbron ◇	13
Mucambo	10
Oropouche	7
Germiston	6
Bhanja	6
Hantaan*	6
Mayaro	5
Spondweni	4
Murray Valley encephalitis	3
Semliki Forest	3 (1 death)
Powassan	2
Dugbe	2
Issyk-kul	1
Koutango	1

* Export permit required by Department of Commerce

◇ An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Large quantities and high concentrations of Semliki Forest virus are commonly used or manipulated by molecular biologists under conditions of moderate or low containment. Although antibodies have been demonstrated in individuals working with this virus, the first overt (and fatal) laboratory-associated infection with this virus was reported in 1979. Because the outcome of this infection may have been influenced by an unusual route of

Agent Summary Statements—Arboviruses/Related Zoonotic Viruses

exposure or high dosage, a compromised host, or a mutated strain of the virus, this case and its outcome are not typical. More recently, SFV was associated with an outbreak of febrile illness among European soldiers stationed in Bangui.⁹ The route of exposure was not determined in the fatal laboratory infection; for the natural infections, mosquitoes were the probable vector. SALS continues to classify SFV as a BSL-3 virus, with the caveat that most activities with this virus can be safely conducted at Biosafety Level 2.

Some viruses (e.g., Akabane, Israel turkey meningoencephalitis) are listed in BSL-3, not because they pose a threat to human health, but because they are exotic diseases of domestic livestock or poultry.

Laboratory Hazards: The agents listed in this group may be present in blood, cerebrospinal fluid, 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) may be relatively stable in dried blood or exudates. For five BSL-3/4 viruses, attenuated strains exist which may be handled at BSL-2, as listed in Table 2.

Recommended Precautions: Biosafety Level 3 practices, safety 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 qualified to enter rooms where the agents or infected animals are present. Indeed, but for this vaccine, the aerosol infectivity and high case fatality of yellow fever virus would make its classification BSL-4. For Venezuelan equine encephalomyelitis, investigational (IND) vaccine TC-83 provides excellent protection against many epizootic strains. This protection may extend to other VEE strains of the complex, including Everglades, Mucambo, Tonate, and

Agent Summary Statements—Arboviruses/Related Zoonotic Viruses

Cabassou viruses. TC-83 vaccine should be used as part of a comprehensive safety program and may be particularly important in protecting those working with infected animals and virus concentrates. The administration of the vaccine and the use of its inactivated counterpart (C-84) should be determined by personnel experienced in the use of these vaccines within the constraints of the IND. Likewise, an inactivated IND vaccine is available for Rift Valley Fever virus, and a live attenuated Junin virus vaccine (Candid #1) is available. These IND vaccines may be obtained from the U.S. Army Medical Research and Materiel Command, after consultation with USAMRIID (telephone 301-619-2833).

SALS has lowered the biohazard classification of Junin virus to BSL-3, provided that all at-risk personnel are immunized and the laboratory is equipped with HEPA-filtered exhaust. SALS has also lowered the biohazard classification of the Central European tick-borne encephalitis (CETBE) viruses to BSL-3, provided all at-risk personnel are immunized. An inactivated IND vaccine for CETBE is available from USAMRIID and is recommended for all laboratory and animal care personnel working with the agent or infected animals, and for all personnel entering laboratories or animal rooms when the agent is in use.

Enhanced Biosafety Level 3 Containment: Situations may arise for which enhancements to Biosafety Level 3 practices and equipment are required. An example of such a situation would be a BSL-3 laboratory performing diagnostic testing on specimens from patients with hemorrhagic fevers thought to be due to dengue or yellow fever. When the origin of these specimens is Africa, the Middle East, or South America, the potential is present for such specimens to contain etiologic agents, such as arenaviruses, filoviruses or other viruses that are usually manipulated in a Biosafety Level 4 laboratory. Enhancements to BSL-3 laboratories might include one or more of the following three categories: a) enhanced respiratory protection of personnel against aerosols; b) HEPA filtration of dedicated exhaust air from the laboratory; c) decontamination of laboratory liquid effluent. Additional appropriate training for all animal care personnel should be considered.

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

Biocontainment of Infectious Unknowns: Decisions regarding biohazard classification for materials containing unidentified infectious virus should be based on all available information regarding the agent. Viruses isolated from infected human patients should be handled at the BSL-3 level with enhanced precautions, as detailed in the hantavirus agent summary, or preferably at BSL-4, unless there is confidence that the agent is not infectious via the aerosol route. All unknown samples should be handled at BSL-3, unless there is evidence of aerosol transmission (which would require BSL-4 containment.) SALS will continue to evaluate infectivity and virulence data for all viruses registered in the *Catalogue of Arboviruses and Certain Other Viruses of Vertebrates* and for newly emerging viruses prior to registration.

Transfer of Agents: For a permit to import these agents, contact CDC.

Table 3. Arboviruses and Certain Other Viruses Assigned to Biosafety Level 3 (on the basis of insufficient experience)

Adelaide River	Cacipacore	Iaco
Agua Preta	Calchaqui	Ibaraki
Alenquer	Cananea	Ife
Almeirim	Caninde	Iguape
Altamira	Chim	Inhangapi
Andasibe	Coastal Plains	Inini
Antequera	Connecticut	Issyk-Kul
Araguari	Corfou	Itaituba
Aransas Bay	Dabakala	Itimirim
Arbia	Douglas	Itupiranga
Arboledas	Enseada	Jacareacanga
Babanki	Estero Real	Jamanxi
Batken	Fomede	Jari
Belem	Forecariah	Kedougou
Berrimah	Fort Sherman	Khasan
Bimbo	Gabek Forest	Kindia
Bobaya	Gadgets Gully	Kyzylgach
Bobia	Garba	Lake Clarendon
Bozo	Gordil	Llano Seco
Buenaventura	Gray Lodge	Macaua
Cabassou ^{a,b}	Gurupi	Mapuera

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

Mboke	Palma	Sena Madureira
Meaban	Para	Sepik
Mojui Dos Compos	Paramushir	Shokwe
Monte Dourado	Paroo River	Slovakia
Munguba	Perinet	Somone
Naranjal	Petevo	Sripur
Nariva	Picola	Tai
Nasoule	Playas	Tamdy
Ndelle	Pueblo Viejo	Telok Forest
New Minto	Purus	Termeil
Ngari	Radi	Thiafora
Ngoupe	Razdan	Tilligerry
Nodamura	Resistencia	Tinaroo
Northway	Rochambeau	Tlacotalpan
Odrenisrou	Salanga	Tonate ^{a,b}
Omo	San Juan	Utinga
Oriximina	Santa Rosa	Xiburema
Ouango	Santarem	Yacaaba
Oubangui	Saraca	Yaounde
Oubi	Saumarez Reef	Yoka
Ourem	Sedlec	Yug Bogdanovac
Palestina		

^a SALS recommends that work with this agent should be conducted only in Biosafety Level 3 facilities which provide for HEPA filtration of all exhaust air prior to discharge from the laboratory.

^b TC-83 vaccine is available and is recommended for all persons working with this agent.

Table 4. Arboviruses and Certain Other Viruses Assigned to Biosafety Level 3

Aino	Dhori	Junin ^{c,d,h}
Akabane ^c	Dobrava-Belgrade	Kairi
Banna ^{a,f}	Dugbe	Kimberley
Bhanja	Everglades ^{c,d}	Koutango
Central Eur. TBE ^{b,d}	Flexal	Kumlinge (Cent. Eur. TBE)
(Kumlinge. Hypr, Hanzalova, Absettarov)	Germiston ^c	Louping III ^{a,c,h}
Chikungunya ^{c,d}	Getah	Mayaro
Cocal	Hantaan ^h	Middelburg
	Israel Turkey mening.	Mobala
	Japanese enc. ^h	

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

Mopeia ^e	Puumala	Thogoto
Mucambo ^{c,d}	Rift Valley fever	Turuna
Murray Valley enc.	^{a,c,d,h} ◊	Venezuelan equine
Nairobi sheep	Rocio ^c	encephalitis
disease ^{a,◊}	Sagiyama	^{c,d,h} ◊
Ndumu	Sal Vieja	Vesicular stomatitis
Negishi	San Perlita	(Alagoas) ^{h,◊}
Oropouche ^c	Semliki Forest	Wesselsbron ^{a,c} ◊
Orungo	Seoul	West Nile
Peaton	Sin Nombre	Yellow fever ^{c,d}
Piry [◊]	Spondweni	Zinga ^g
Powassan	St. Louis enc.	

- ^a The importation, possession, or use of this agent is restricted by USDA regulation or administrative policy. See Appendix D.
- ^b Central European Tick Borne Encephalitis virus (CETBE) is not a registered name in *The International Catalogue of Arboviruses-1985*. Until the registration issue has been 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. These four viruses are antigenically homogeneous and are distinguishable from Russian Spring-Summer encephalitis (RSSE) virus.^{10,11,12} While there is a vaccine available which confers immunity to the CETBE group of genetically (>98%) homogeneous viruses, the efficacy of this vaccine against RSSE virus infections has not been established. Thus, SALS has reclassified the CETBE group of viruses as Biosafety Level 3 when personnel are immunized with CETBE vaccine. RSSE remains classified as a Biosafety Level 4 virus.
- ^c SALS recommends that work with this agent be conducted only in Biosafety Level 3 facilities which provide for HEPA filtration of all exhaust air prior to discharge from the laboratory.
- ^d A vaccine is available and is recommended for all persons working with this agent.
- ^e This virus is presently being registered in the *Catalogue of Arboviruses*.

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

- ^f Scientists from the People’s Republic of China have verbally reported Banna virus to be associated with severe human cases of encephalitis. Translations of the original publications from Chinese into English were not available for this revision.
- ^g Zinga is now recognized to be identical with Rift Valley Fever virus.
- ^h Export permit required from Department of Commerce.
- ◇ An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

Arboviruses, Arenaviruses, and Filoviruses Assigned to Biosafety Level 4

SALS recommends that work with the 11 arboviruses, arenaviruses, or filoviruses¹³ included in Table 5 be conducted at the equivalent of Biosafety Level 4 practices, safety equipment, and facilities. These recommendations 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 Biosafety Level 4 agents (e.g., Russian Spring-Summer Encephalitis virus) also be provisionally handled at this level until sufficient laboratory experience indicates their retention at this level or movement to work at a lower level. As noted above, with immunization, SALS recommends downgrading the biohazard classification of Junin virus and the Central European Tick-borne encephalitis virus complex viruses (Absettarov, Hanzalova, Hypr, and Kumlinge) to BSL-3. Laboratory or laboratory animal-associated infections have been reported with the following agents:^{14,15,16,17,18,19,20}

<u>Virus</u>	<u>Cases (SALS)</u>
Junin*	21 (1 death)
Marburg*	25 (5 deaths)
Russian Spring-Summer	8
Congo-Crimean hemorrhagic	8 (1 death)
Omsk hemorrhagic fever	5
Lassa*	2 (1 death)
Machupo*	1 (1 death)

Agent Summary Statements—Arboviruses/Related Zoonotic Viruses

<u>Virus</u>	<u>Cases (SALS)</u>
Ebola*	1
Sabia*	3 (1 death)

* Export permit required by Department of Commerce.

Rodents are natural reservoirs of Lassa virus (*Mastomys* spp.), Junin, and Machupo viruses (*Calomys* spp.), Guanarito (*Zygodontomys* spp.), and perhaps other members of this group. Nonhuman primates were associated with the initial outbreaks of Kyasanur Forest disease (*Presbytis* spp.) and Marburg disease (*Cercopithecus* spp.). More recently, filoviruses related to Ebola were associated with *Macaca* spp. and chimpanzees (*Pan troglodytes*). Arthropods are the natural vectors of the tick-borne encephalitis complex agents. Work with or exposure to rodents, nonhuman primates, or vectors naturally or experimentally infected with these agents represents a potential source of human infection.

Laboratory Hazards. The infectious agents may be present in blood, urine, respiratory and throat secretions, semen, and tissues from human or animal hosts, and in arthropods, rodents, and nonhuman primates. 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.^{21,22}

Recommended Precautions. Biosafety Level 4 practices 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 Biosafety Level 4 maximum containment facility.^{23,24}

Transfer of Agent: For a permit to import these agents, contact CDC. Contact the Department of Commerce for a permit to export these agents. Laboratory registration with CDC is required before sending or receiving these select agents.

Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

Table 5. Arboviruses, Arenaviruses and Filoviruses Assigned to Biosafety Level 4

Central European tick-borne encephalitis
Congo-Crimean hemorrhagic fever
Ebola
Guanarito
Junin
Kyasanur Forest disease
Lassa
Machupo
Marburg
Omsk hemorrhagic fever
Russian Spring-Summer encephalitis
Sabia

Transfer of agents: For a permit to import these agents, contact CDC. Contact the Department of Commerce for a permit to export these agents. Laboratory registration with CDC is required before sending or receiving these select agents.

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Agent Summary Statements–Arboviruses/Related Zoonotic Viruses

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APPENDIX A

Primary Containment: Biological Safety Cabinets

Biological Safety Cabinets (BSCs) are among the most effective and the most commonly used primary containment devices in laboratories working with infectious agents.¹The three general types available (Class I, II, III) have performance characteristics and applications which are described in this appendix.

Properly maintained Class I and II BSCs, when used in conjunction with good microbiological techniques, provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents). Both Class I and II BSCs have inward face velocities (75-100 linear feet per minute) that provide comparable levels of containment to protect laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSCs also protect the research material itself through high-efficiency particulate air filtration (HEPA filtration) of the air flow down across the work surface (vertical laminar flow). Class III cabinets offer the maximum protection to laboratory personnel, the community, and the environment because all hazardous materials are contained in a totally enclosed, ventilated cabinet.

CLASS I

(Note: Class I BSCs are currently being manufactured on a limited basis; many have been replaced by Class II BSCs.)

The *Class I Biological Safety Cabinet* (Fig. 1) is a negative-pressure, ventilated cabinet usually operated with an open front and a minimum face velocity at the work opening of at least 75 linear feet per minute (lfpm). All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory or to the outside. The Class I BSC is designed for general microbiological research with low- and moderate-risk agents, and is useful for containment of mixers, blenders, and other equipment. These cabinets are *not* appropriate for handling research materials that are vulnerable to airborne contamination, since the inward flow of

Appendix A

unfiltered air from the laboratory can carry microbial contaminants into the cabinet.

The Class I BSC can also be used with an installed front closure panel without gloves, which will increase the inward flow velocity to approximately 150 lfpm. If such equipped cabinets are ducted to the outside exhaust, they may be used for toxic or radiolabelled materials used as an adjunct to microbiological research. Additionally, arm-length rubber gloves may be attached to the front panel with an inlet air pressure release for further protection. In this configuration, it is necessary to install a make-up air inlet fitted with a HEPA filter in the cabinet

CLASS II

The *Class II Biological Safety Cabinet* (Fig. 2) is designed with inward air flow at a velocity to protect personnel (75-100 lfpm), HEPA-filtered downward vertical laminar airflow for product protection, and HEPA-filtered exhaust air for environmental protection. Design, construction, and performance standards for Class II BSCs, as well as a list of products that meet these standards, have been developed by and are available from the National Sanitation Foundation International,²Ann Arbor, Michigan. Utilization of this standard and list should be the first step in selection and procurement of a Class II BSC.

Class II BSCs are classified into two types (A and B) based on construction, air flow velocities and patterns, and exhaust systems. Basically, Type A cabinets are suitable for microbiological research *in the absence of* volatile or toxic chemicals and radionuclides, since air is recirculated within the cabinet. Type A cabinets may be exhausted into the laboratory or to the outdoors via a "thimble" connection to the building exhaust system.

Type B cabinets are further sub-typed into types B1, B2, and B3. A comparison of the design features and applications are presented in Figures 2b, 2c, and 2d, respectively. Type B cabinets are hard-ducted to the building exhaust system and contain negative pressure plenums. These features, plus a face

Appendix A

velocity of 100 fpm, allow work to be done with toxic chemicals or radionuclides.

It is imperative that Class I and II biological safety cabinets be tested and certified *in situ* at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not supersede the critical certification prior to use in the laboratory.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the biological safety cabinets. Of particular note are activities that may disrupt the inward directional airflow. Repeated insertion and withdrawal of the workers' arms into and out of the work chamber, opening and closing doors to the laboratory or isolation cubicle, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is in use have been demonstrated to cause the escape of aerosolized particles from within the cabinet. Class I and II cabinets should be located away from traffic patterns and doors. Air flow from fans, room air supply louvers and other air moving devices can disrupt the airflow pattern at the face of the cabinet. Strict adherence to recommended practices for the use of BSCs and their proper placement in the laboratory are as important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself.

CLASS III

The *Class III Biological Safety Cabinet* (Fig. 3) is a totally enclosed, ventilated cabinet of gas-tight construction and offers the highest degree of personnel and environmental protection from infectious aerosols, as well as protection of research materials from microbiological contaminants. Class III cabinets are most suitable for work with hazardous agents that require Biosafety Level 3 or 4 containment.

Appendix A

All operations in the work area of the cabinet are performed through attached arm length rubber gloves or half-suits. The Class III cabinet is operated under negative pressure. Supply air is HEPA-filtered and the cabinet exhaust air is filtered through two HEPA filters in series, or HEPA filtration followed by incineration, before discharge outside of the facility.

All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet system. The Class III cabinet must be connected to a double-doored autoclave and/or chemical dunk tank used to sterilize or disinfect all materials exiting the cabinet, and to allow supplies to enter the cabinet. Several Class III cabinets are therefore typically set up as an interconnected system.

POSITIVE-PRESSURE PERSONNEL SUIT

Personnel protection equivalent to that provided by Class III cabinets can also be obtained with the use of a one-piece, ventilated suit worn by the laboratory worker when working with Biosafety Level 3 or 4 agents in a "suit area" and using Class I or II BSCs. The personnel suit is maintained under positive pressure with a life-support system to prevent leakage into the suit. In this containment system, the worker is isolated from the work materials.

The personnel suit area must be essentially equivalent to a large Class III cabinet. The area is entered through an air-lock fitted with airtight doors. A chemical shower is provided as a "dunk tank" to decontaminate the surfaces of the suit as the worker leaves the area. The exhaust air from the suit area is filtered through two HEPA filter units installed in series. The entire area must be under negative pressure.

As with Class III BSCs, the gloves of the personnel suit are the most vulnerable component of the system, as they are subject to punctures by sharps or animal bites.

Other Devices: Horizontal laminar flow "clean benches" are used in clinical, pharmaceutical, and laboratory facilities strictly for

Appendix A

product protection. This equipment must *never* be used for handling toxic, infectious, radioactive, or sensitizing materials, since the worker sits in the immediate downstream exhaust from the "clean bench." Vertical laminar flow benches may be useful for certain manipulations of clean materials (e.g., pouring agar plates) but should not be used when working with infectious materials.

References:

1. U.S. Department of Health and Human Services. *Primary Containment of Biohazards: Selection, Installation and Use of Biological Safety Cabinets*. (Washington: GPO, 1995)
2. National Sanitation Foundation Standard 49. 1983. Class II (Laminar Flow) Biohazard Cabinetry. Ann Arbor, Michigan.

Table 1. Comparison of Biological Safety Cabinets

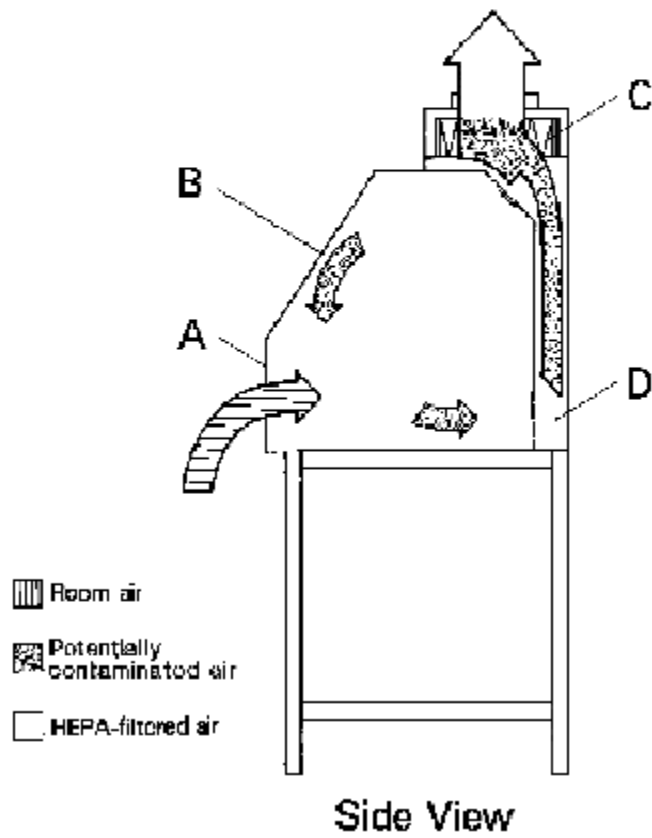
Type	Face velocity (1fpm)	Airflow Pattern	Radionuclides/ Toxic Chemicals	Biosafety Level(s)	Product Protection
Class I* open front	75	In at front; rear and top through HEPA filter	No	2,3	No
Class II Type A	75	70% recirculated through HEPA; exhaust through HEPA	No	2,3	Yes
Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Yes (Low levels/volatility)	2,3	Yes
Type B2	100	No recirculation; total exhaust via HEPA and hard ducted	Yes	2,3	Yes
Type B3	100	Same as IIA, but plenum under negative pressure to room and exhaust air is ducted	Yes	2,3	Yes
Class III	NA	Supply air inlets and exhaust through 2 HEPA filter	Yes	3,4	Yes

Appendix A

Figure 1. Class I Biological Safety Cabinet

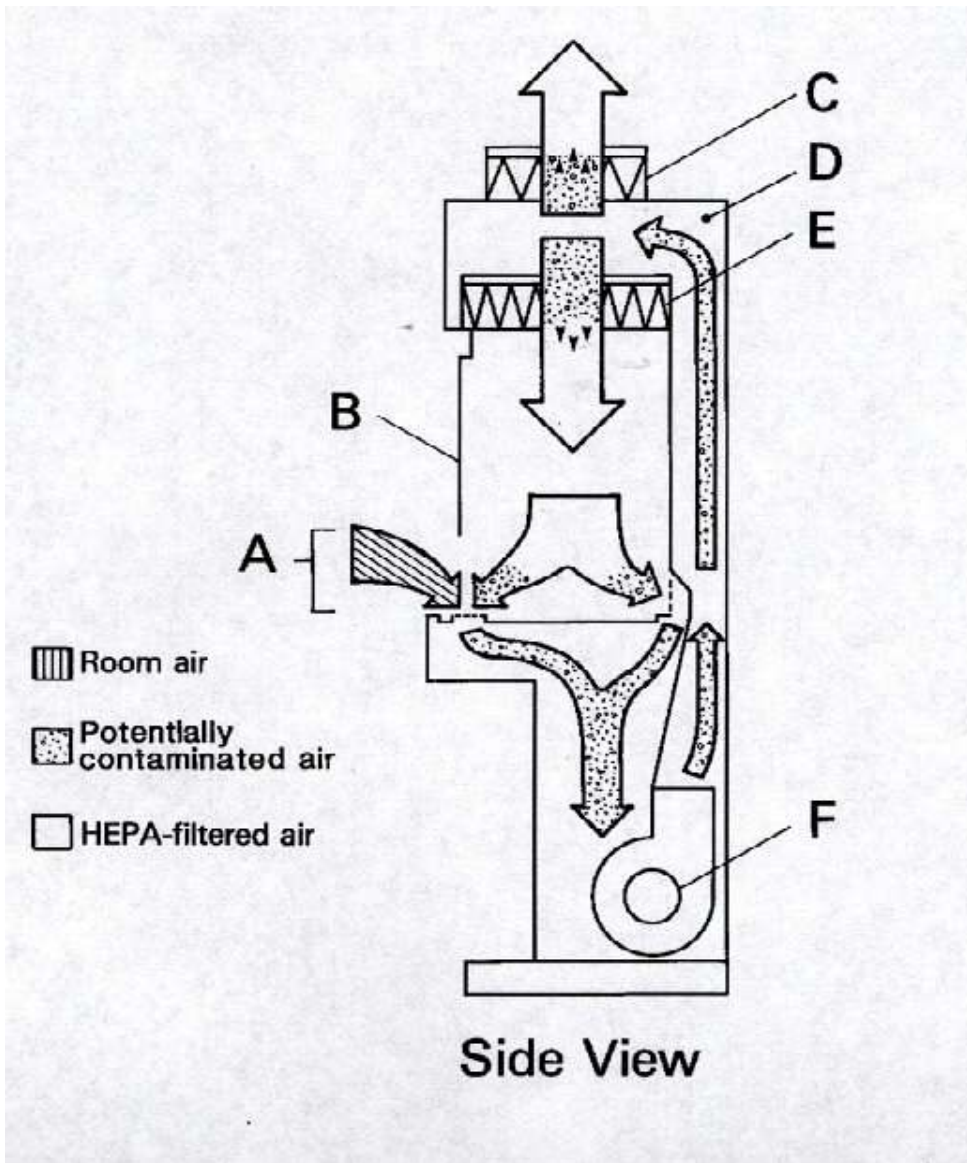
Figure 1.
Class I Biological Safety Cabinet.

A. front opening; B. sash; C. exhaust HEPA filter; D. exhaust plenum



Appendix A

Figure 2a. Class II, Type A Biological Safety Cabinet



Appendix A

Figure 2b. Class II, Type B1 Biological Safety Cabinet

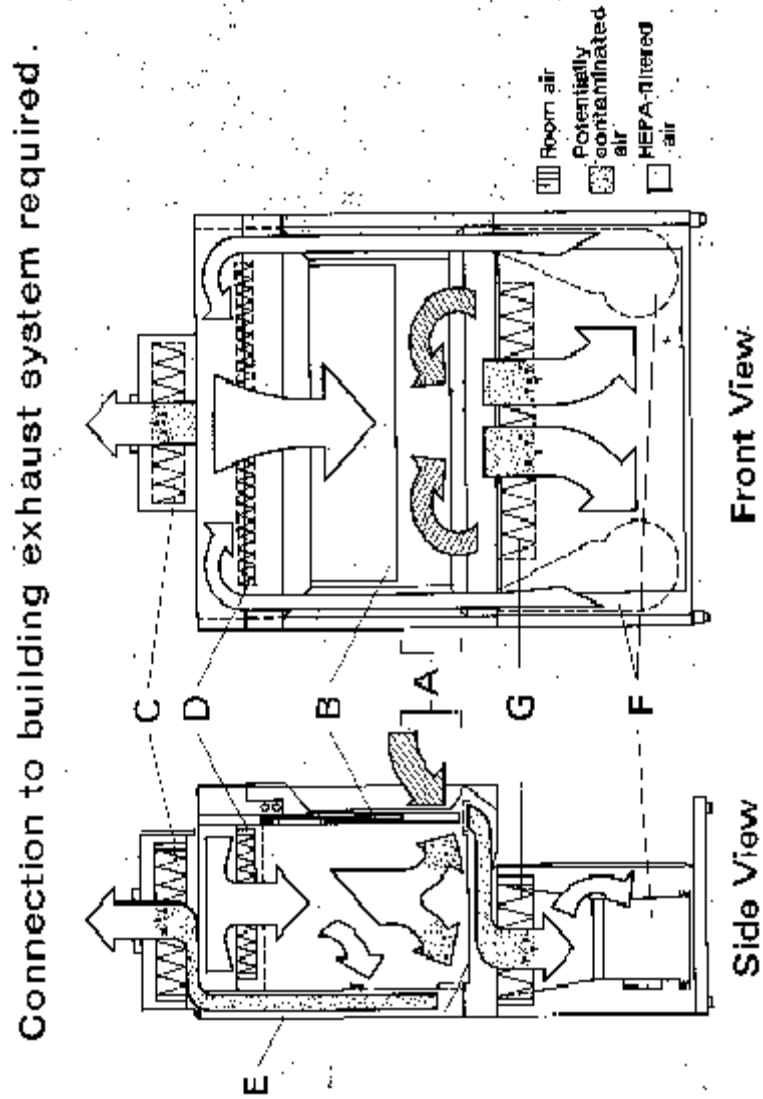
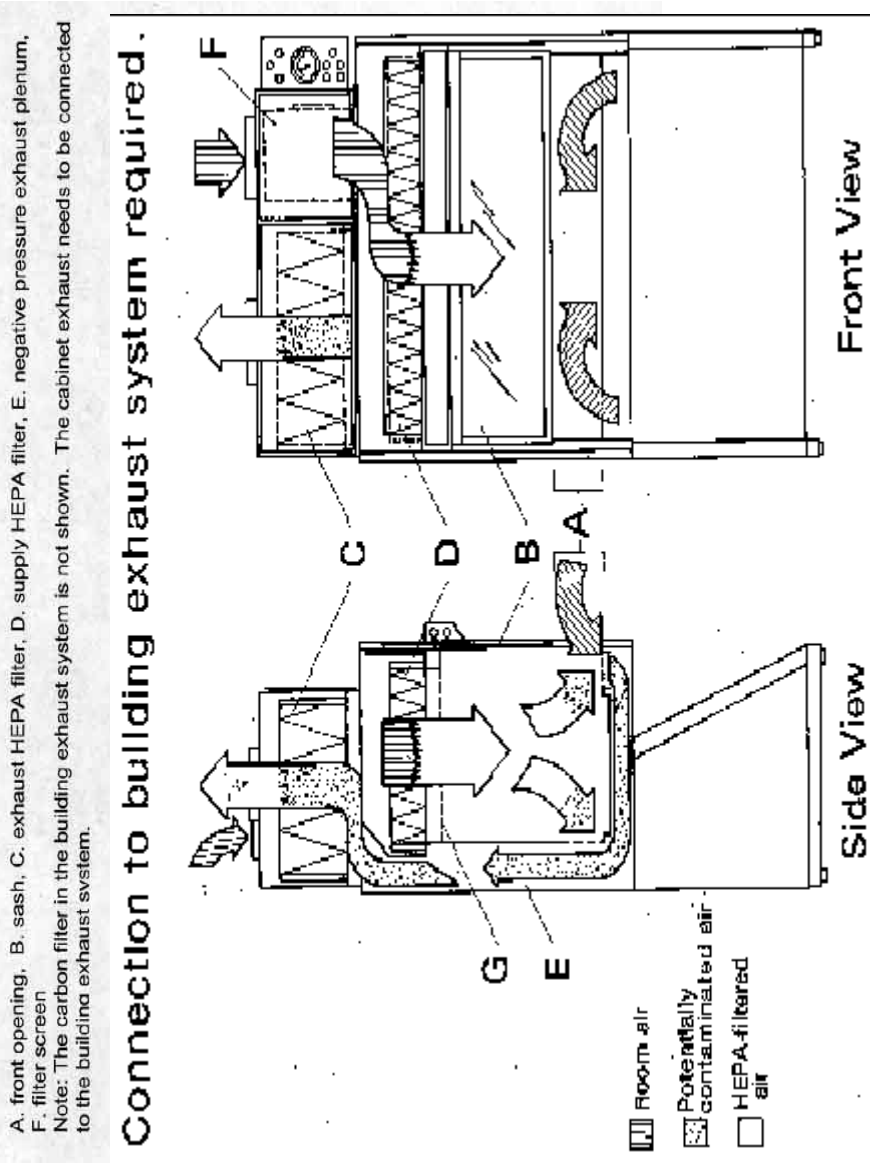
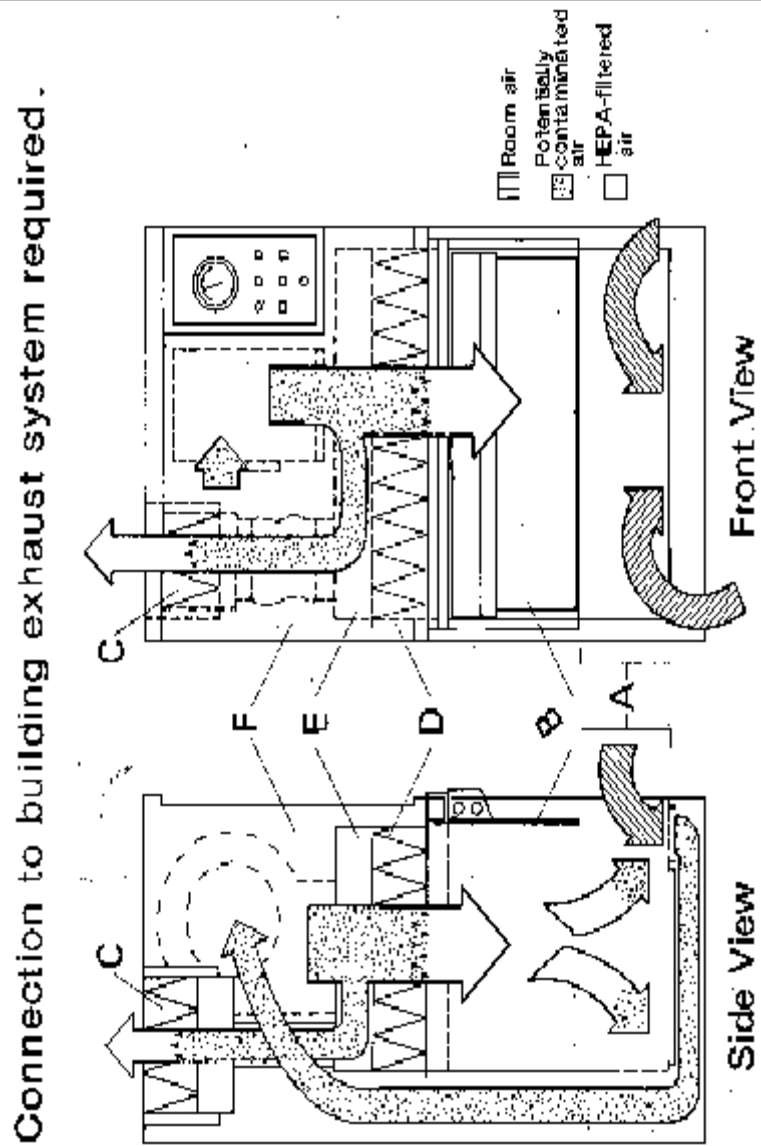


Figure 2c. Class II, Type B2 Biological Safety Cabinet



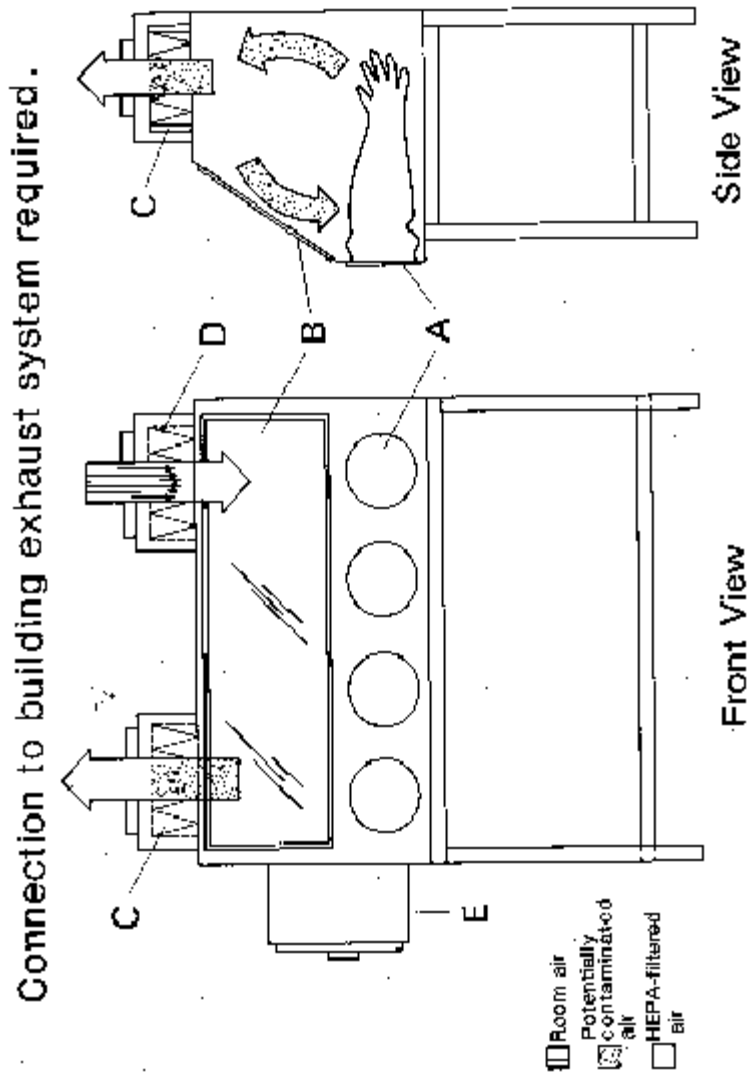
Appendix A

Figure 2d. Table-top Model



Appendix A

Figure 3. Class III Biological Safety Cabinet



APPENDIX B

Immunoprophylaxis

An additional level of protection for at-risk personnel may be achieved with appropriate prophylactic immunizations. A written organizational policy that defines at-risk personnel, specifies risks as well as benefits of specific vaccines, and distinguishes between required and recommended vaccines is essential. In developing such an organizational policy, these recommendations and requirements should be specifically targeted at infectious diseases known or likely to be encountered in a particular facility.

Licensed vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risks (e.g., local or systemic reactions) should be required for all clearly identified at-risk personnel. Examples of such preparations include vaccines against hepatitis B, yellow fever, rabies, and poliomyelitis. Recommendations for giving less efficacious vaccines, those associated with high rates of local or systemic reactions, those that produce increasingly severe reactions with repeated use, and unlicensed vaccines given under investigational new drug (IND) protocols, should be carefully considered. Products with these characteristics (e.g., cholera, anthrax, and tularemia vaccines) may be recommended but ordinarily should not be required for employment. A complete record of vaccines received on the basis of occupational requirements or recommendations should be maintained in each employee's permanent medical file.

Recommendations for the use of vaccines, adapted from those of the Public Health Service Advisory Committee on Immunization Practices, are included in the agent summary statements in Section VII and are elaborated in the references below.^{1,2} The reader is advised to consult the current related recommendations of the ACIP published in the *CDC Morbidity and Mortality Weekly Report* (MMWR). Particular attention must be given to individuals who are or may become immunocompromised, as recommendations for vaccine administration may be different than for immunologically competent adults.

References:

1. Centers for Disease Control and Prevention. General Recommendations on Immunization Recommendations of the Advisory Committee on Immunization Practices (ACIP) , January 28, 1994 / Morbidity and Mortality Weekly Report (MMWR) 43(RR01);1-38.
2. Centers for Disease Control and Prevention. 1997. Immunization of Health-Care Workers: Recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Hospital Infection Control Practices Advisory Committee (HICPAC) Morbidity and Mortality Weekly Report (MMWR) **46** (RR-18);1-42.

APPENDIX C

Transportation and Transfer of Biological Agents

Biological agents include infectious agents of humans, plants, and animals, as well as the toxins that may be produced by microbes and by genetic material potentially hazardous by itself or when introduced into a suitable vector. Etiologic agents and infectious substances are closely related terms that are found in the transfer and transportation regulations. Biological agents may exist as purified and concentrated cultures but may also be present in a variety of materials such as body fluids, tissues, soil samples, etc. Biological agents and the materials that are known or suspected to contain them are recognized by federal and state governments as hazardous materials and their transportation and transfer is subject to regulatory control.

Transportation refers to the packaging and shipping of these materials by air, land, or sea, generally by a commercial conveyance. *Transfer* refers to the process of exchanging these materials between facilities.

Transportation

Regulations on the transportation of biological agents are aimed at ensuring that the public and the workers in the transportation chain are protected from exposure to any agent that might be in the package. Protection is achieved through (a) the requirements for rigorous packaging that will withstand rough handling and contain all liquid material within the package without leakage to the outside, (b) appropriate labeling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents of the package, (c) documentation of the hazardous contents of the package should such information be necessary in an emergency situation, and (d) training of workers in the transportation chain to familiarize them with the hazardous contents so as to be able to respond to emergency situations.

Appendix C

Regulations

Public Health Service 42 CFR Part 72. Interstate Shipment of Etiologic Agents. This regulation is in revision to harmonize it with the other U.S. and international regulations. A copy of the current regulation may be obtained from the Internet at:

<http://www.cdc.gov/od/ohs>

Department of Transportation. 49 CFR Parts 171-178. Hazardous Materials Regulations.

Applies to the shipment of both biological agents and clinical specimens. Information may be obtained from the Internet at:

<http://www.dot.gov/rules.html>

United States Postal Service. 39 CFR Part 111. Mailability of Etiologic Agents. Codified in the Domestic Mail Manual 124.38: Etiologic Agent Preparations. A copy of the Domestic Mail Manual may be obtained from the Government Printing Office by calling 1-202-512-1800 or from the Internet at:

<http://www.access.gpo.gov>

Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030. Occupational Exposure to Bloodborne Pathogens. Provides minimal packaging and labeling requirements for transport of blood and body fluids within the laboratory and outside of it. Information may be obtained from your local OSHA office or from the Internet:

<http://osha.gov>

Dangerous Goods Regulations (DGR). International Air Transport Association (IATA). These regulations provide packaging and labeling requirements for infectious substances and materials, as well as clinical specimens that have a low probability of containing an infectious substance. These are the regulations followed by the airlines. These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air which is provided by the International Civil Aviation Organization (ICAO). A copy of the

Appendix C

DGR may be obtained by calling 1-800-716-6326 or through the Internet at:

<http://www.iata.org>, or <http://www.who.org>

General Packaging Requirements for Transport of Biological Agents and Clinical Specimens

Figure 1 shows the generalized “triple” (primary receptacle, water tight secondary packaging, durable outer packaging) packaging required for a biological agent of human disease or materials that are known or suspected of containing them. This packaging requires the “Infectious Substance” label shown in Figure 2 on the outside of the package. This packaging must be certified to meet rigorous performance tests as outlined in the DOT, USPS, PHS, and IATA regulations.

Clinical specimens with a low probability of containing an infectious agent are also required to be “triple” packaged, but performance tests require only that the package shall not leak after a four-foot drop test. DOT, PHS, and IATA require a “clinical specimen” label on the outside of the package.

Transfer

Regulations on the transfer of biological agents are aimed at ensuring that the change in possession of biological materials is within the best interests of the public and the nation. These regulations require documentation of the personnel, facilities, and justification of need for the biological agent in the transfer process and subsequent approval of the transfer process by a federal authority. The following regulations fit in this category:

Importation of Etiologic Agents of Human Disease

42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiologic Agents, Hosts and Vectors. This regulation requires an import permit from the Centers for Disease Control and Prevention for importing etiologic agents of human disease and any materials, including live animals or insects, that may contain them. An

Appendix C

application and information on importation permits may be obtained by calling 1-888-CDC-FAXX and enter document number 101000 or on the Internet at:

<http://www.cdc.gov/od/ohs/biosfty/imp rtp er.htm l>

Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases

9 CFR Parts 92, 94, 95 96, 122 and 130. These regulations requires an import permit from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services to import or domestically transfer etiologic agents of livestock, poultry, other animals, and any materials that might contain these etiologic agents. Information may be obtained at (301) 734-3277, or from the Internet at:

<http://aphisweb.aphis.usda.gov/ncie>.

Importation of Plant Pests

7 CFR Part 330. *Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone and Quarry Products; Garbage*. This regulation requires a permit to import or domestically transfer a plant pest, plant biological agent, or any material that might contain them. Information can be obtained by calling 301-734-3277 or through the Internet at:

<http://www.aphis.us da.g ov./ppq/ppqperm its.htm l>.

Transfer of Select Biological Agents of Human Disease

42 CFR Part 72.6 *Additional Requirements for Facilities Transferring or Receiving Select Agents*. Facilities transferring or receiving select agents must be registered with the CDC and each transfer of a select agent must be documented. Information may be obtained on the Internet at:

<http://www.cdc.gov/od/ohs/lrsat.htm>

Appendix C

Department of Commerce. 15 CFR Parts 730 to 799. This regulation requires that exporters of a wide variety of etiologic agents of human, plant and animal diseases, including genetic material, and products which might be used for culture of large amounts of agents, will require an export license. Information may be obtained by calling the DoC Bureau of Export Administration at 202-482-4811 or through the Internet at: <http://bxa.fedworld.gov>, or <http://www.bxa.doc.gov>

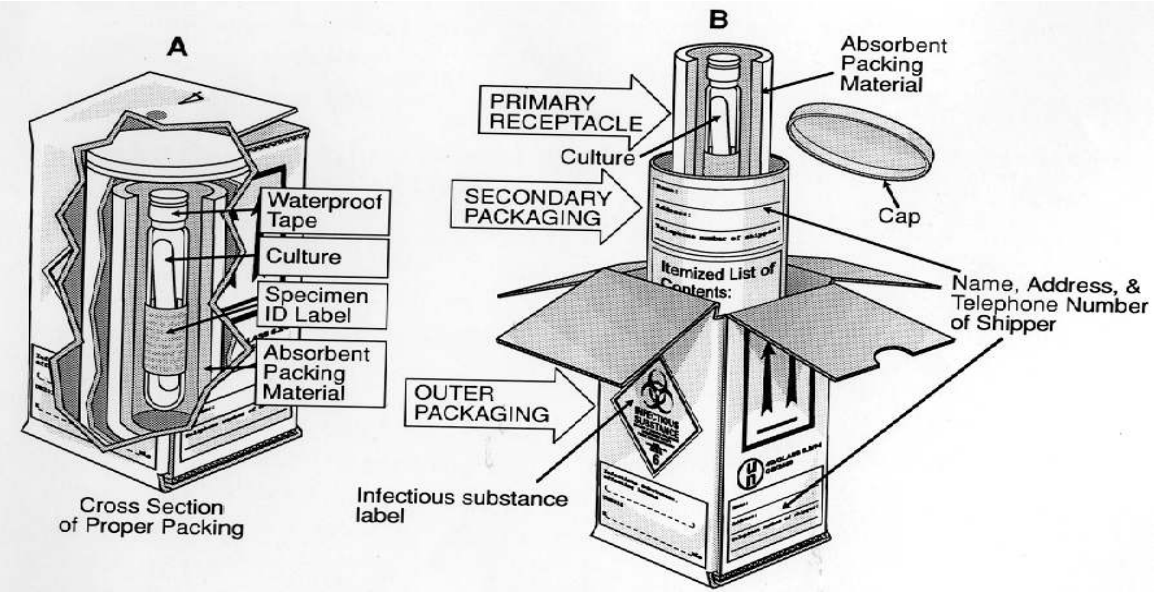
Figures 1 and 2 illustrate the packaging and labeling of infectious substances and clinical specimens in volumes of less than 50 ml. in accordance with the provisions of subparagraph 72.3(a) of the regulation on Interstate Shipment of Etiologic Agents (42 CFR, Part 72). A revision is pending that may result in additional package labeling requirements, but this has not been issued in final form as of the publication of this fourth edition of BMBL.

For further information on any provision of this regulation contact:

Centers for Disease Control and Prevention
Attn: External Activities Program
Mail Stop F-05
1600 Clifton Road N.E.
Atlanta, GA 30333
Telephone: (404) 639-4418
FAX: (404) 639-2294

Note that the shipper's name, address and telephone number must be on the outer and inner containers. The reader is also advised to refer to additional provisions of the Department of Transportation (49 CFR, Parts 171-180) Hazardous Materials Regulations.

Figure 1. Packaging and Labeling of Infectious Substances A



APPENDIX D

Restricted Animal Pathogens

Nonindigenous pathogens of domestic livestock and poultry may require special laboratory design, operation, and containment features not generally addressed in this publication. The importation, possession, or use of the following agents is prohibited or restricted by law or by U.S. Department of Agriculture regulations or administrative policies:

African horse sickness	<i>Mycoplasma agalactiae</i>
African Swine fever virus*	<i>Mycoplasma mycoides (mycoides)</i>
Akabane virus	Nairobi sheep disease virus
Avian influenza virus	(Ganjam virus)
<i>Besnoitia besnoiti</i>	Newcastle disease virus*
Bluetongue virus*	(velogenic strains)
Borna disease virus	Peste des petits ruminants*
Bovine spongiform	(plague of small ruminants)
encephalopathy	Rift Valley fever virus*
Bovine infectious petechial	Rinderpest virus*
fever agent	Sheep and goat pox*
<i>Brucella abortus</i>	Swine vesicular disease virus*
<i>Brucellosis melitensis</i> *	Teschen disease virus*
<i>Burkholderia mallei (Pseudomonas</i>	<i>Theileria annulata</i>
<i>mallei – Glanders)</i>	<i>Theileria lawrencei</i>
Camelpox virus	<i>Theileria bovis</i>
Classical swine fever	<i>Theileria hirci</i>
<i>Cochliomyia hominivorax</i>	<i>Trypanosoma brucei</i>
(Screwworm)	<i>Trypanosoma congolense</i>
<i>Cowdria ruminantium</i> (heartwater)	<i>Trypanosoma equiperdum</i>
Creutzfeldt-Jacob Disease variant,	(dourine)
Bovine spongiform	<i>Trypanosoma evansi</i>
encephalopathy	<i>Trypanosoma vivax</i>
Ephemeral fever virus	Venezuelan equine
Foot and mouth disease virus*	encephalomyelitis
<i>Histoplasma (Zymonema)</i>	Vesicular exanthema virus
<i>farciminosum</i>	Vesicular stomatitis
Louping ill virus	Viral hemorrhagic disease of
Lumpy skin disease virus	rabbits
<i>Mycobacterium bovis</i>	Wesselsbron disease virus

* Export license required by Department of Commerce.

Appendix D

The importation, possession, use, or interstate shipment of animal pathogens other than those listed above may also be subject to regulations of the U.S. Department of Agriculture.

A USDA/APHIS import permit is required to import any infectious agent of animals that is listed by USDA/APHIS as a restricted animal pathogen. Such a permit may be required to import any other infectious agent of livestock or poultry. An import permit is also required to import any livestock or poultry animal product such as blood, serum, or other tissues.

Additional information may be obtained by writing to:

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services, National Center for Import and Export
4700 River Road, Unit #40
Riverdale, Maryland 20737-1231

Telephone: (301) 734-3277
Fax: (301) 734-8226

Internet: <http://www.aphis.usda.gov/ncei>

APPENDIX E

Resources for Information

Resources for information, consultation, and advice on biohazard control, decontamination procedures, and other aspects of laboratory safety management include:

Centers for Disease Control and Prevention
Attention: External Activities Program
Atlanta, Georgia 30333
Telephone: (404) 639-4418

National Institutes of Health
Attention: Division of Safety
Bethesda, Maryland 20205
Telephone: (301) 496-1357

National Animal Disease Center
U.S. Department of Agriculture
Ames, Iowa 50010
Telephone: (515) 663-7258

United States Department of Labor, Occupational Safety and Health Administration

- Occupational Exposure to Blood borne Pathogens, Final Rule. Fed Reg 1991; 56: 64041-64182
http://www.osha-slc.gov/OshStd_data/1910_1030.html.
- Proposed rule for TB:
http://www.osha-slc.gov/FedReg_osh_data/FED19980205.html
- Occupational Safety and Health Standards. 29 CFR Part 1910: (http://www.osha-slc.gov/OshStd_toc/OSHA_Std_toc_1910.html)

Appendix E

Guidelines:

Centers for Disease Control and Prevention

- *Tuberculosis:*
1994: <http://www.cdc.gov/nchstp/tb/pubs/250001.pdf>
1997(labs): <http://www.cdc.gov/od/ohs/tb/tbdoc2.html>
- *Immunization for Health Care Workers:*
<http://www.cdc.gov/epo/mmwrr/preview/rr4618.html>
- *Guidelines for Infection Control in Healthcare Personnel,*
1998:
http://www.cdc.gov/ncidod/hip/draft_gu/waisgate.txt
- *HIV Prophylaxis:*
http://www.cdc.gov/epo/mmwrr/mmwrr_r.html

Department of the Army, DOD. 32 CFR Parts 626, 627 –
Biological Defense Safety Program: (<http://www.gpo.gov>)

National Committee for Clinical Laboratory Standards (NCCLS)

- *Approved guidelines for protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue.* Dec. 1977, NCCLS Doc. M29-A (ISBN1-56238-339-6. [Http://www.lexoffice@nccls.org](http://www.lexoffice@nccls.org)).

National Institutes of Health:

- NIH Guidelines for Recombinant DNA Molecules:
<http://www.NIH.gov/od/orda/toc.html>
- NIH Office of Recombinant DNA Activities:
<http://www.NIH.gov/od/orda>

Appendix F - Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents

December 6, 2002 Prepared by
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¹Office of the Director
Office of Health and Safety (Retired)
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Office of Health and Safety

Summary

In recent years, concern has increased regarding use of biologic materials as agents of terrorism, but these same agents are often necessary tools in clinical and research microbiology laboratories. Traditional biosafety guidelines for laboratories have emphasized use of optimal work practices, appropriate containment equipment, well-designed facilities, and administrative controls to minimize risk of worker injury and to ensure safeguards against laboratory contamination.

The guidelines discussed in this report were first published in 1999 (U.S. Department of Health and Human Services/CDC and National Institutes of Health. Biosafety in microbiological and biomedical laboratories [BMBL]. Richmond JY, McKinney RW, eds. 4th ed. Washington, DC: US Department of Health and Human Services, 1999 [Appendix F]). In that report, physical security concerns were addressed, and efforts were focused on preventing unauthorized entry to laboratory areas and preventing unauthorized removal of dangerous biologic agents from the laboratory. Appendix F of BMBL is now being revised to include additional information regarding personnel, risk assessments, and inventory controls. The guidelines contained in this report are intended for laboratories working with select agents under biosafety-level 2, 3, or 4 conditions as described in Sections II and III of BMBL. These recommendations include conducting facility risk assessments and developing comprehensive security plans to minimize the probability of misuse of select agents.

Risk assessments should include systematic, site-specific reviews of 1) physical security; 2) security of data and electronic technology systems; 3) employee security; 4) access controls to laboratory and animal areas; 5) procedures for agent inventory and accountability; 6) shipping/transfer and receiving of select agents; 7) unintentional incident and injury policies; 8) emergency response plans; and 9) policies that address breaches in security. The security plan should be an integral part of daily operations. All employees should be well-trained and equipped, and the plan should be reviewed annually, at least.

Introduction

Traditional laboratory biosafety guidelines have emphasized use of optimal work practices, appropriate containment equipment, well-designed facilities, and administrative controls to minimize risks of unintentional infection or injury for laboratory workers and to prevent contamination of the outside environment (1). Although clinical and research microbiology laboratories might contain dangerous biologic, chemical, and radioactive materials, to date, only a limited number of reports have been published of materials being used intentionally to injure laboratory workers or others (2–7). However, recently, concern has increased regarding possible use of biologic, chemical, and radioactive materials as terrorism agents (8,9). In the United States, recent terrorism incidents (10) have resulted in the substantial enhancement of existing regulations

and creation of new regulations governing laboratory security to prevent such incidents.

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002* (the Act) required institutions to notify the U.S. Department of Health and Human Services (DHHS) or the U.S. Department of Agriculture (USDA) of the possession of specific pathogens or toxins (i.e., select agents[†]), as defined by DHHS, or certain animal and plant pathogens or toxins (i.e., high-consequence pathogens), as defined by USDA. The Act provides for expanded regulatory oversight of these agents and a process for limiting access to them to persons who have a legitimate need to handle or use such agents. The Act also requires specified federal agencies to

The material in this report originated in the Office of Health and Safety, Robert H. Hill, Jr., Ph.D., Acting Director.

* Public Law 107–188, June 12, 2002.

[†] Throughout this report, the term *select agent* refers to specifically regulated pathogens and toxins as defined in Title 42, Code of Federal Regulations (CFR), Part 73, including pathogens and toxins regulated by both DHHS and USDA (i.e., overlapping agents and toxins). The reader should note that 42 CFR Part 73 has not been published yet, and is still under federal review with anticipated publication in December 2002.

withhold from public disclosure, among other requirements, site-specific information regarding the identification of persons, the nature and location of agents present in a facility, and the local security mechanisms in use. In addition, the Uniting and Strengthening America by Providing Appropriate Tools Required To Intercept and Obstruct Terrorism (USA PATRIOT) Act of 2001[§] prohibits restricted persons from shipping, possessing, or receiving select agents. Violation of either of these statutes carries criminal penalties.

Appendix F of the 4th edition of the CDC/National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories (BMBL) was the first edition to address laboratory security concerns (1). However, that publication primarily addressed physical security concerns (e.g., preventing unauthorized entry to laboratory areas and preventing unauthorized removal of dangerous biologic agents from the laboratory). The guidelines presented here are provided to assist facility managers with meeting the regulatory mandate of 42 Code of Federal Regulation (CFR) 73 and, therefore, include information regarding personnel, risk assessments, and inventory controls. These guidelines are intended for laboratories where select agents are used under biosafety levels (BSL) 2, 3, or 4 as described in Sections II and III of BMBL. Appendix F of BMBL is being revised to include consideration of the following biosecurity policies and procedures:

- risk and threat assessment;
- facility security plans;
- physical security;
- data and electronic technology systems;
- security policies for personnel;
- policies regarding accessing the laboratory and animal areas;
- specimen accountability;
- receipt of agents into the laboratory;
- transfer or shipping of select agents from the laboratory;
- emergency response plans; and
- reporting of incidents, unintentional injuries, and security breaches.

Definitions

Biosafety: Development and implementation of administrative policies, work practices, facility design, and safety equipment to prevent transmission of biologic agents to workers, other persons, and the environment.

Biosecurity: Protection of high-consequence microbial agents and toxins, or critical relevant information, against theft or diversion by those who intend to pursue intentional misuse.

Biologic Terrorism: Use of biologic agents or toxins (e.g., pathogenic organisms that affect humans, animals, or plants) for terrorist purposes.

Responsible official: A facility official who has been designated the responsibility and authority to ensure that the requirements of Title 42, CFR, Part 73, are met.

Risk: A measure of the potential loss of a specific biologic agent of concern, on the basis of the probability of occurrence of an adversary event, effectiveness of protection, and consequence of loss.

Select agent: Specifically regulated pathogens and toxins as defined in Title 42, CFR, Part 73, including pathogens and toxins regulated by both DHHS and USDA (i.e., overlapping agents or toxins).

Threat: The capability of an adversary, coupled with intentions, to undertake malevolent actions.

Threat assessment: A judgment, based on available information, of the actual or potential threat of malevolent action.

Vulnerability: An exploitable capability, security weakness, or deficiency at a facility. Exploitable capabilities or weaknesses are those inherent in the design or layout of the biologic laboratory and its protection, or those existing because of the failure to meet or maintain prescribed security standards when evaluated against defined threats.

Vulnerability assessment: A systematic evaluation process in which qualitative and quantitative techniques are applied to arrive at an effectiveness level for a security system to protect biologic laboratories and operations from specifically defined acts that can oppose or harm a person's interest.

Risk Assessment

Recommendation: Conduct a risk assessment and threat analysis of the facility as a precursor to the security plan.

Background: In April 1998, the General Accounting Office issued a report regarding terrorism (11). A key finding of that report was that threat and risk assessments are widely recognized as valid decision-support tools for establishing and prioritizing security program requirements. A threat analysis, the first step in determining risk, identifies and evaluates each threat on the basis of different factors (e.g., the capability and intent to attack an asset, the likelihood of a successful attack, and the attack's probable lethality). Risk management is the deliberate process of understanding risk (i.e., the likelihood that a threat will harm an asset with certain severity of

[§] Public Law 107-56, October 26, 2001.

consequences) and deciding on and implementing actions to reduce that risk. Risk management principles are based on acknowledgment that 1) although risk usually cannot be eliminated, it can be reduced by enhancing protection from validated and credible threats; 2) although threats are possible, certain threats are more probable than others; and 3) all assets are not equally critical. Therefore, each facility should implement certain measures to enhance security regarding select agents. The following actions should assist decision-makers in implementing this recommendation:

- Each facility should conduct a risk assessment and threat analysis of its assets and select agents. The threat should be defined against the vulnerabilities of the laboratory to determine the necessary components of a facility security plan and system (12,13).
- The risk assessment should include a systematic approach in which threats are defined and vulnerabilities are examined; risks associated with those vulnerabilities are mitigated with a security systems approach (12,13).
- Ensure the security plan includes collaboration between senior management, scientific staff, human resource officials, information technology (IT) staff, engineering officials, and security officials. This coordinated approach is critical to ensuring that security recommendations provide a reasonable and adequate assurance of laboratory security without unduly impacting the scientific work.

Facility Security Plans

Recommendation: Establish a facility security plan.

- Each facility should develop a comprehensive security plan that complies with 42 CFR Part 73 and reviews the need for policies in
 - physical security;
 - data and IT system security;
 - security policies for personnel;
 - policies for accessing select agent areas;
 - specimen accountability;
 - receipt of select agents into the laboratory;
 - transfer or shipping of select agents from the laboratory;
 - emergency response plans; and
 - reporting of incidents, injuries, and breaches.
- Develop security policies based on site-specific assessments. Security plans should include measures that address physical security of building and laboratory areas. Policies should also address concerns associated with access, use, storage, and transfer of sensitive data. If sensitive electronic data are present, IT specialists should assess the security of hardware and software

products in addition to the security of local area networks.

- Review safety, security, and IT policies and procedures at least annually for consistency and applicability. These procedures should also be reviewed after any incident or change in regulations. Necessary changes should be incorporated into the revised plans and communicated to all.
- Laboratory supervisors should ensure that all laboratory workers and visitors understand security requirements and that all employees are trained and equipped to follow established procedures. The security plan should be an integral part of daily operations. New employees should receive training when they first begin work, and all employees should receive training at least annually thereafter. Training should be updated as policies and procedures change. All training should be documented by maintaining records of training schedules and employee attendance.
- Security plans should receive periodic performance testing to determine their effectiveness. Test procedures can vary from a simple check of keys, locks, and alarms to a full-scale laboratory or facility exercise.

Security Policies for Personnel

Recommendation: Establish security-related policies for all personnel.

- Honest, reliable, and conscientious workers represent the foundation of an effective security program. Facility administrators and laboratory directors should be familiar with all laboratory workers.
- Establish a policy for screening employees who require access to select agent areas to include full- and part-time employees, contractors, emergency personnel, and visitors. Additional screening might be necessary for employees who require access to other types of sensitive or secure data and work areas. These screening procedures should be commensurate with the sensitivity of the data and work areas (e.g., federal security clearances for government employees and contractors).
- Ensure that all workers approved for access to select agents (e.g., students, research scientists, and other short-term employees) wear visible identification badges that include, at a minimum, a photograph, the wearer's name, and an expiration date. Facility administrators should consider using easily recognizable marks on the identification badges to indicate access to sensitive or secure areas.

Access Control

Recommendation: Control access to areas where select agents are used or stored.

- Consolidate laboratory work areas to the greatest extent possible to implement security measures more effectively. Separate select agent areas from the public areas of the buildings. Lock all select agent areas when unoccupied. Use keys or other security devices to permit entry into these areas.
- Methods of secure access and monitoring controls can include key or electronic locking pass keys, combination key pad, use of lock-boxes to store materials in freezers or refrigerators, video surveillance cameras, or other control requirements. Protocols for periodically changing combination keypad access numbers should be developed.
- Assess the need for graded levels of security protection on the basis of site-specific risk and threat analysis. This security can be accomplished through card access systems, biometrics, or other systems that provide restricted access.
- Lock all freezers, refrigerators, cabinets, and other containers where select agents are stored when they are not in direct view of a laboratory worker.
- Limit access to select agent areas to authorized personnel who have been cleared by the U.S. Department of Justice as indicated in 42 CFR Part 73. All others entering select agent areas must be escorted and monitored by authorized personnel.
- Record all entries into these areas, including entries by visitors, maintenance workers, service workers, and others needing one-time or occasional entry.
- Limit routine cleaning, maintenance, and repairs to hours when authorized employees are present and able to serve as escorts and monitors.
- Establish procedures and training for admitting repair personnel or other contractors who require repetitive or emergency access to select agent areas.
- Ensure visitors are issued identification badges, including name and expiration date, and escorted and monitored into and out of select agent areas. Such visits should be kept to a minimum.
- Ensure procedures are in place for reporting and removing unauthorized persons. These procedures should be developed through collaboration among senior scientific, administrative, and security management personnel. These procedures should be included in security training and reviewed for compliance at least annually.

Select Agent Accountability

Recommendation: Establish a system of accountability for select agents.

- Establish an accounting procedure to ensure adequate control of select agents and maintain up-to-date inventory of seed stocks, toxins, and agents in long-term storage. Records should include data regarding the agent's location, use, storage method, inventory, external transfers (sender/receiver, transfer date, and amount), internal transfer (sender/receiver, transfer date, amount), further distribution, and destruction (method, amount, date, and a point of contact).
- Establish procedures that maintain accurate and up-to-date records of authorizations for entry into limited access areas (i.e., a current list of persons who possess door keys and those who have knowledge of keypad access numbers or the security system).

Receiving Select Agents

Recommendation: Develop procedures for bringing select agent specimens into the laboratory.

- A centralized receiving area for select agents is recommended to maximize safety and minimize security hazards associated with damaged or unknown packages.
- Facilities should establish procedures for inspecting all packages (i.e., by visual or noninvasive techniques) before they are brought into the laboratory area. Suspicious packages should be handled as prescribed by federal and state law enforcement agencies.
- Biologic safety cabinet or other appropriate containment device should be used when opening packages containing specimens, bacterial or virus isolates, or toxins. Packages should be opened by trained, authorized personnel.

Transfer or Shipping of Select Agents

Recommendation: Develop procedures for transferring or shipping select agents from the laboratory.

- Package, label, and transport select agents in conformance with all applicable local, federal, and international transportation and shipping regulations, including U.S. Department of Transportation (DOT) regulations.[§] Materials that are transported by airline carrier should also comply with packaging and shipping regulations set by

[§] U.S. Department of Transportation, Research and Special Programs Administration, 49 CFR, Parts 171–180.

the International Air Transport Association (IATA). Personnel who package, handle, and ship these agents (including import and export) should be subject to all applicable training. The responsible facility official should be notified of all select agent transfers, internal or external.

- Ensure required permits (e.g., granted by the U.S. Public Health Service, USDA, DOT, U.S. Department of Commerce, and IATA) are obtained before select agents are prepared for transport. Standard operating procedures should be in place for import and export activities.
- Decontaminate contaminated or possibly contaminated materials before they leave the laboratory area.
- Avoid hand-carrying select agents when transferring them to other external facilities. If select agents are to be hand-carried on common carriers, all applicable packaging, transport, and training regulations should be followed.
- Develop and follow a protocol for intrafacility transfer of all select agents.

Emergency Response Plans

Recommendation: Implement an emergency response plan.

- Limiting access to select agent laboratory and animal areas can make implementing an emergency response more difficult. This should be considered as emergency plans are developed.
- Evaluate select agent laboratory and animal areas for safety and security concerns before an emergency plan is developed.
- Develop and integrate laboratory emergency plans with facilitywide plans. These plans should also include such adverse event assessments as bomb threats, severe weather (e.g., hurricanes or floods), earthquakes, power outages, and other natural or man-made disasters.
- Include facility administrators, scientific directors, principal investigators, laboratory workers, maintenance and engineering support staff, facility safety officers, and facility security officials in emergency planning.
- Include provisions for immediate notification of and response by laboratory and animal directors, laboratory workers, safety office personnel, or other knowledgeable persons when an emergency occurs.
- Establish advance coordination with local police, fire, and other emergency responders to assist community emergency responders in planning for emergencies in select agent laboratory and animal areas. Discussion should address security concerns associated with sharing of sensitive information regarding secure work areas.

- Consider circumstances that might require the emergency relocation of select agents to another secure location.
- Reevaluate and train employees and conduct exercises of the emergency response plan at least annually.

Incident Reporting

Recommendation: Establish a protocol for reporting adverse incidents.

- Ensure that laboratory directors, in cooperation with facility safety, security, and public relations officials, have policies and procedures in place for reporting and investigating unintentional injuries, incidents (e.g., unauthorized personnel in restricted areas, missing biologic agents or toxins, and unusual or threatening phone calls), or breaches in security measures.
- DHHS or USDA should be notified immediately if select agents are discovered to be missing, released outside the laboratory, involved in worker exposures or infections, or misused. Additionally, all incidents involving select agents (e.g., occupational exposure or breaches of primary containment) should be reported to local and state public health authorities.

Acknowledgments

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APPENDIX G

Integrated Pest Management

Pest management is an important part of managing a research facility. Many pests, such as flies and cockroaches, can mechanically vector disease pathogens and compromise the research environment. Even the presence of innocuous insects can contribute to the perception of unsanitary conditions.

The most common approach to pest control has been the application of pesticides, either as a preventive or remedial measure. Pesticidal treatments can be effective and may be necessary as a corrective measure, but they have limited long-term effect when used alone. Pesticidal applications also present the potential to contaminate the research environment through pesticide drift and volatilization.

To control pests and minimize the use of pesticides, it is necessary to employ a comprehensive program approach to pest management that integrates housekeeping, maintenance, and pest control services. This method of pest control is often referred to as Integrated Pest Management (IPM). The primary goal of an IPM program is to prevent pest problems by managing the facility environment in such a way as to make it less conducive to pest infestation. Along with limited applications of pesticides to control pests, pest control is achieved through proactive operational and administrative intervention strategies to correct conditions that foster pest problems.

IPM is a strategy-based service. The decision to implement an IPM program should be based not only on the cost of the services, but on the effectiveness of the program's components. IPM is site-specific, and each program should be tailored to the environment where it is applied. IPM services in a laboratory will be different from those in an office building or an animal care facility.

Integrated pest management programs can be delineated into various interrelated components which contribute to the

Appendix G

“environmental management” approach to controlling pests.
These are:

- *Facility Design:* The inclusion of pest management issues and requirements in a facility’s planning, design, and construction provides an opportunity to incorporate features that help to exclude pests, minimize pest habitat, and promote proper sanitation. This can help to reduce the need for future corrective pest management services that can be disruptive to research operations.
- *Monitoring:* Traps, visual inspections, and staff interviews are used to identify areas and conditions that may foster pest activity. Monitoring is the central activity of an IPM program and is used in place of preventive pesticidal treatments.
- *Sanitation and Facility Maintenance:* Many pest problems can be prevented or corrected by using proper sanitation, reducing clutter and pest habitat, and by performing repairs that exclude pests and reduce pest habitat. Maintaining records of structural deficiencies and housekeeping conditions can help to track problems and determine if corrective actions are completed in a timely manner.
- *Communication:* A staff member can be designated to meet with pest management personnel to assist in resolving facility issues that impact on pest management. Information on pest activity, and recommendations on personnel practices and facility conditions that impact pest management, can be relayed verbally and in writing to that person. Training on subjects such as pest identification, biology, and sanitation can also promote understanding and cooperation with the goals of the IPM program.
- *Record Keeping:* A logbook can be used to record pest activity and conditions pertinent to the IPM program. It may contain protocols and procedures for IPM services in that facility; Material Safety Data Sheets on pesticides; pesticide labels; treatment records; floor plans; survey reports; etc.

Appendix G

- *Nonpesticidal Pest Control:* Pest control methods such as trapping, exclusion, caulking, washing, and freezing can be applied safely and effectively when used in conjunction with proper sanitation and structural repair.
- *Pest Control With Pesticides:* Preventive applications of pesticides should be discouraged, and treatments should be restricted to areas of known pest activity. When pesticides are applied, the least toxic product(s) available should be used and applied in the most effective and safe manner.
- *Program Evaluation and Quality Assurance:* Quality assurance and program review should be performed to provide an objective, ongoing evaluation of IPM activities and effectiveness. This is to ensure that the program is controlling pests and meeting the specific needs of the facility program(s) and its occupants. Based upon this review, current pest management protocols can be modified and new procedures implemented.
- *Technical Expertise:* A qualified entomologist can provide helpful technical guidance in developing and implementing an IPM program. Pest management personnel should be licensed and certified through examination by the appropriate regulatory agency.
- *Safety:* By limiting the scope of pesticidal treatments and using nonpesticidal control practices, IPM can minimize the potential of pesticide exposure to the research environment and the staff.

Prior to initiating any type of pest management program, development of an operational framework for IPM services can help to promote collaboration between pest management specialists and facility personnel. This framework can also be used to incorporate facility restrictions and operational and procedural issues into the IPM program. An effective pest management program is an integral part of the facility's management. Including an IPM policy statement in the facility's

Appendix G

standard operating procedures can increase awareness of the program .

Training on the principles and practices of structural (indoor) integrated pest management and information on IPM programs is available from many sources. Some of these are university entomology departments, county extension offices, the Entomological Society of America, state departments of agriculture, state pest control associations, the National Pest Control Association, suppliers of pest control equipment, and pest management consultants or pest management firms. There are also correspondence courses available from several universities as well as short courses and training conferences on structural pest management.

Additional Information:

Urban Entomology. 1996. Insect and Mite Pests in the Human Environment. W.H. Robinson. Chapman and Hall. New York.

Advances in Urban Pest Management. 1986. Gary W. Bennett and John M. Owens, eds. Van Nostrand Reinhold Company. New York,

Common Sense Pest Control. 1991. Least-toxic solutions for your home, garden, pests and community. William Olkowski, Sheila Daar, Helga Olkowski. The Taunton Press., Inc.,

Internet:

- National Pest Control Association: <http://www.pestworld.org>
- Biocontrol Network: <http://www.bioconet.com>

APPENDIX H

Working With Human and Other Primate Cells and Tissues

The Centers for Disease Control and Prevention and the National Institutes of Health wish to express their appreciation to Frank P. Simone, M.S., and Jane Caputo, B.A., of the American Type Culture Collection (ATCC), a global bioscience organization dedicated to biological standards and biodiversity, for their contributions to the preparation of this Appendix.

At least 24 documented cases of infection of laboratory workers handling primary cell cultures (e.g., primary rhesus monkey kidney cells) have occurred in the past 30 years.^{1,2} While a limited number of laboratory-associated infections have been reported as resulting from the handling of human and other primate cells, there is a more significant risk to acquiring infection with HBV or HIV from exposure to human blood and other body fluids,^{3,4,5} and OSHA has developed a bloodborne pathogens standard.⁶ Procedures have been published to reduce contamination of cell cultures with microorganisms^{7,8} or other cells.⁹

Potential Laboratory Hazards. The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV and HIV, as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissues. Other primate cells and tissues also present risks to laboratory workers.¹⁰ Potential hazards to laboratory workers are presented by cells transformed with viral agents, such as SV-40, EBV, or HBV, as well as cells carrying viral genomic material. Tumorigenic human cells also are potential hazards as a result of self-inoculation.¹¹

Recommended Practices. Human and other primate cells should be handled using Biosafety Level 2 practices and containment. All work should be performed in a biosafety cabinet, and all material should be decontaminated by autoclaving or disinfection before discarding.^{12,13,14,15} All employees working with human cells and tissues should be enrolled in the institutional Bloodborne Pathogens Program, and should work under the

policies and guidelines established by the institutions' Exposure Control Plan.¹⁶ Employees should provide a baseline serum sample, be offered hepatitis B immunization, and be evaluated by a health care professional following an exposure incident.

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16. Occupational Exposure to Bloodborne Pathogens (6)

APPENDIX I

Guidelines for Work With Toxins of Biological Origin

In recognition of the growing number of microbiological and biomedical laboratories working with toxins of biological origin, the following is provided as a guideline for working with these toxins.

The material below is adapted from the Biological Defense Safety Program, Technical Safety Requirements (DA Pamphlet 385-69)¹, and Appendix A of the United States Department of Labor Occupational Safety and Health Association rule "Occupational Exposure to Hazardous Chemicals in Laboratories"².

Laboratory managers and facility safety officials are encouraged to utilize the references listed below and to consult with subject matter experts before using any toxin, to ensure that appropriate facilities, containment equipment, policies and procedures, personnel training programs and medical surveillance protocols specific to the toxin and the laboratory are in place.

General

The laboratory facilities, equipment, and procedures appropriate for work with toxins of biological origin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed. If both toxins and infectious agents are used, both must be considered when containment equipment is selected and policies and procedures are written. If animals are used, animal safety practices must also be considered.

Standard Practices

Standard practices listed under BSL 2 and BSL 3 (pages 20 and 27) should be reviewed and incorporated as appropriate into protocols for work with toxins.

Appendix I

Special Practices

Special practices listed under BSL 2 and BSL 3 (pages 21 and 28) should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. Each laboratory should develop a chemical hygiene plan specific to the toxin(s) used in that laboratory. The chemical hygiene plan should 1) identify the hazards that will be encountered in normal use of the toxin, and those that could be encountered in case of a spill or other accident, and 2) specify the policies and practices to be used to minimize risks (e.g., containment and personal protective equipment, management of spills, management of accidental exposures, medical surveillance).³
2. Training specific to the toxin(s) used should be required and documented for all laboratory personnel working with toxins, before starting work with the toxin and at intervals thereafter.
3. An inventory control system should be in place.
4. Toxins should be stored in locked storage rooms, cabinets, or freezers when not in use.
5. Access to areas containing toxins should be restricted to those whose work assignments require access.
6. Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins should be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by the safety officer. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.
7. The user should verify inward airflow of the hood or biological safety cabinet before initiating work.

Appendix I

8. All work should be done within the operationally effective zone of the hood or biological safety cabinet.
9. When toxins are in use, the room should be posted to indicate "Toxins in Use—Authorized Personnel Only." Any special entry requirements should be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while toxins are in use.
10. All high risk operations should be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
11. Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container should be decontaminated and placed in a clean secondary container. Toxins should be transported only in leak/spill-proof secondary containers.
12. Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g., used gloves) should be disposed of as toxic waste. Materials contaminated with infectious agents as well as toxins should also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.
13. The interior of the hood, glove box, or cabinet should be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the hood, box, or cabinet should be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

Appendix I

Safety Equipment

Appendix I

The safety equipment guidelines listed under BSL 2 and BSL 3 (see Section III) should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered.
2. Eye protection should be worn if an open-fronted containment system is used.
3. Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. For example, use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.
4. When handling dry forms of toxins that are electrostatic:
 - a. Do not wear gloves (such as latex) that help to generate static electricity
 - b. Use glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.
5. When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin.
6. Consider both toxin and diluent when selecting gloves and other protective clothing.
7. If infectious agents and toxins are used together in an experimental system, consider both when selecting protective clothing and equipment.

Appendix I

Laboratory Facilities

Laboratory facility recommendations listed under BSL 2 and BSL 3 (See Section III) and OSHA standards⁴ should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. Vacuum lines. When vacuum lines are used with systems containing toxins, they should be protected with a HEPA filter to prevent entry of toxins into the lines. Sink drains should be similarly protected when water aspirators are used.

References

1. Department of the Army, DOD. 32 CFR Parts 626, 627 – Biological Defense Safety Program.
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INDEX

Index

African Horse sickness	220
African Swine Fever virus	220
Akabane virus	220
Allergic reactions	131
Animal Biosafety Levels (ABSL)	54
Animal Biosafety Level 1	54
Animal Biosafety Level 2	57, 88, 90, 93-97, 99, 101, 103, 104, 106, 108, 109, 111, 118-122, 150, 156, 158, 164, 165
Animal Biosafety Level 3	62, 91, 98, 149, 164
Animal Biosafety Level 4	69
Animal facilities	5, 14, 53, 54
Animal pathogens	16, 220, 221, 224
Anthrax	88, 212
Arboviruses and Arenaviruses	186, 189
Assigned to Biosafety Level 2	20, 183, 186
Assigned to Biosafety Level 3	27, 189, 193, 194
Assigned to Biosafety Level 4	37, 196, 197
Armadillos	102, 103
Ascaris spp.	131, 132
B virus (herpesvirus simiae)	3, 158
Bacillus anthracis	88
Bacillus subtilis	11
Bacterial agents	88
Besnoitia besnoiti	220
Biological Safety Cabinets (BSC)	9, 200
Biological Safety Cabinets (BSCs)	
Class I	9, 15, 52, 75, 105, 120, 167, 200-203, 205, 206
Class II	9, 15, 24, 32, 35, 36, 42, 50, 68, 120, 200, 201, 205, 207-209
Class III	9, 14, 32, 35, 36, 41, 42, 44-46, 52, 68, 69, 72, 73, 75, 156, 160, 200, 202, 203, 205, 211, 240
Positive-pressure Personnel Suit	75, 203
Biosafety levels (BSL)	
Biosafety Level 1	5, 10-12, 17, 19, 54
Biosafety Level 2	12, 15, 19, 20, 27, 57, 86, 88, 90-112, 118-123, 127, 129-132, 136, 138, 148, 150, 153, 156-159, 162-165, 167, 168, 174, 176, 183-186, 191, 200, 234

Biosafety Level 3	13, 26, 27, 30, 36, 62, 69, 86, 88, 90-92, 94, 95, 98, 102, 106, 107, 109, 112, 119, 121, 136, 138, 148-150, 158, 159, 164, 168, 176, 185, 189, 191-195, 202, 203
Biosafety Level 4	13, 14, 36-38, 41, 42, 46, 50, 69, 73, 160, 192, 195-197
Bioterrorism	ix
Blastomyces dermatitidis	118
Bloodborne pathogens	4, 12, 15, 158, 170, 171, 215, 222, 234, 235
BMBL	3, 3, 4, 218
Bordetella pertussis	89
Borna disease virus	220
Botulism	94, 95
Bovine	viii, 135, 138, 139, 174, 175, 220
spongiform encephalopathy	viii, 135, 137-139, 174, 175
Brucella	1, 90, 91, 220
abortus	90, 220
canis	90
melitensis	90, 91, 220
suis	90
Brucellosis	1, 2, 90, 91, 220
BSC	9, 13, 14, 24, 75, 160, 200-202
C virus	157
Cabinet laboratory	22, 29, 39, 42, 52, 58, 63, 70, 73, 75
Calomys spp.	197
Camelpox virus	220
Campylobacter	92, 99
coli	92, 97
fetus	92, 101, 129, 161, 164
jejuni	92
Cercopithecus spp.	197
Cestode parasites	130
taenia solium	130
Chimpanzees	156, 158, 197
Chlamydia	93
pneumoniae	93
psittaci	93, 94
trachomatis	93, 94
Cholera	1, 111, 212
Clean	31, 34, 45, 49, 53, 65, 70, 203, 204, 239
Clinical laboratories	15, 53, 98, 105, 108, 109

Clostridium	
botulinum	94, 95
tetani	95, 96
Clostridium	94, 95
Coccidioides immitis	118
Coccidioidomycosis	118, 119
Cochliomyia hominivorax	220
Congo-Crimean hemorrhagic fever	14, 198
Conidia	118, 120, 121, 123
Containment	viii, 5, 8-10, 12, 15, 17, 18, 20, 24, 26, 27, 29, 31, 33, 36, 42-44, 47, 49, 52, 57, 60, 62, 66, 69, 73, 75, 82, 85, 86, 88, 90-112, 127, 129, 132, 149, 154, 155, 157, 158, 162-166, 168, 171, 175, 183, 185, 190, 192, 193, 197, 200, 202, 203, 220, 224, 226, 234, 237, 238, 240
Primary containment	8, 10, 12, 66, 73, 90, 92, 95, 102, 106, 107, 112, 127, 129, 132, 157, 162, 164, 168, 175, 200
Secondary containment	36, 85
Coxiella burnetii	13, 148, 149
Creutzfeldt-Jakob	136-139, 174, 175
Cryptococcus neoformans	119
Cysticercus cellulosae	130, 131
Cytomegalovirus	161
Decontamination	10, 13, 23, 31, 33, 34, 36, 40, 42-50, 52, 67-69, 75, 76, 137, 141, 172, 192, 222, 239
Dengue	183, 185, 186, 192
Dermatophytes	122
Diagnostic specimens	16, 163
Diphtheria	84, 95, 96
Dirty	44, 53
Ebola	77, 196-198
Encephalitis	
Russian Spring-Summer	2, 13, 154, 155, 158, 190, 192, 195-198
Ephemeral fever virus	220
Epidermophyton	122
Equine encephalomyelitis	184, 185, 189-191
Escherichia coli	97
Cytotoxin-producing (VTEC/SLT) organisms	97
Facility design	8-13, 17, 36, 46, 50, 69, 231
Fasciola spp.	129
Filoviruses	192, 196, 197
Foot and mouth disease	220

Francisella tularensis	98
Fungal agents	118
Ganjam virus	220
Giardia spp.	128
Gloves	10, 13, 17, 19, 20, 25, 27, 32, 39, 52, 55, 59, 60, 64, 65, 72, 75, 92, 94, 100, 101, 106, 110, 112, 122, 127, 130, 131, 138, 140-143, 156, 158, 160, 168-170, 175, 176, 185, 201, 203, 239, 240
Gonorrhoeae	106
Guidelines	3, 3-5, 11, 15, 76, 77, 80, 81, 83, 136, 138, 153, 156, 160, 170, 172, 173, 223, 224, 234, 235, 237, 240
Hanson et al	2
Hantaviruses	153, 193
Harrington and Shannon	2
Heartwater	220
Helicobacter pylori	99
Hendra virus	154, 155
HEPA filter	45, 46, 49, 50, 68, 200, 201, 203, 205, 241
Hepatitis	2, 4, 11, 12, 15, 22, 29, 58, 63, 77, 84, 87, 137, 148, 156-158, 163, 170, 212, 234, 235
A virus	156
B virus	4, 12, 15, 77, 84, 87, 157, 158, 170, 234
C virus	157
D virus	157
E virus	156
Herpesvirus simiae	
CHV-1	158-160
Herpesviruses	81, 161
Herpesvirus simiae	3, 158-161
Human Herpesviruses	161
Histoplasma	
capsulatum	120
farcinosum	220
Human Immunodeficiency Virus	4, 15, 77, 170, 234
Hypr	194-196
Imm unoprophylaxis	84, 212
Importation and interstate shipment	16
Infectious canine hepatitis virus	11
Influenza	84, 154, 162, 220
International transfer	ix
Investigational New Drug	84, 95, 212
Junin virus	79, 192, 196

Kuru	134-137, 173, 174
Kyasanur Forest disease	197, 198
Laboratory coats	19, 24, 52, 56, 60, 65, 75, 130, 160
Laboratory hazards	84, 88-112, 118, 120-123, 127-132, 148, 150, 153, 155-157, 159, 161-163, 165, 167, 168, 170, 174, 176, 184, 185, 191, 197, 234
Laboratory Practice	8
Laboratory Security and Emergency Response	224
Legionella pneumophila	101, 102
Leishmania spp.	127
Leprosy	102, 103
Leptospira interrogans	99
Leptospirosis	99
Listeria monocytogenes	100, 101
Louping ill virus	220
Lumpy skin disease virus	220
Lymphocytic choriomeningitis	163, 190
Lymphogranuloma venereum (LGV)	93
Macaque	158, 160, 169
Machupo	196-198
Malaria	127
Marburg	3, 14, 77, 196-198
Mask	24
Medical Waste Tracking Act of 1988	4
Metacercaria	129, 130
Meyer and Eddie	1
Mice	93, 94, 102, 105, 106, 120-122, 135-137, 139, 163-165, 176
Microsporium	122
Molds	122, 143
Monkey pox	167
Multi-drug-resistant strains	4
Mycobacterium	13, 102-104, 220, 234
asiaticum	104
avium complex	104
bovis	103, 104, 106, 220
chelonei	104
fortuitum	104
kansasii	103, 104
leprae	102-104
malmoense	104
marinum	104

scrofulaceum	104
simiae	3, 104, 158-161
szulgai	104
tuberculosis	1, 2, 4, 13, 15, 84, 103-106, 223, 234
ulcerans	104
xenopi	104
Mycoplasma agalactiae	220
Mycoplasma mycoides	220
Naegleria	
fowleri	128, 129
gruberi	11
Naegleria	11, 128, 129
Nairobi sheep disease	194, 220
Needles and syringes	22, 30, 40, 59, 63, 71
Neisseria	106, 107
gonorrhoeae	106
meningitidis	107
Newcastle disease virus	220
Omsk hemorrhagic fever	196, 198
Paramyxovirus	154
Parasitic agents	127
Blood and Tissue Protozoal Parasites of Humans	127
Cestode	130, 131
Intestinal	128, 131
Nematode	131
Trematode	129, 130
Pathogens . iii, 4, 12, 15, 16, 30, 38, 82, 136, 138, 158, 161, 170,	
171, 215, 220-222, 224, 230, 234, 235	
Penicillium marneffii	118, 123
Personal protective equipment . 10, 21, 33, 60, 65, 79, 159, 160,	
238	
Personnel suits	36, 42
Pest management	230-233
Peste des petits ruminants	220
Pipetting	1, 18, 20, 28, 91
Plague	111, 220
Plasmodium spp.	127
Plastic-backed paper toweling	31
Poliovirus	84, 164-166
Pontiac fever	101
Poxviruses	166, 167
Presbytis spp.	197

Primates . . .	56, 60, 103, 105, 106, 109, 131, 150, 156, 158, 160, 163, 165, 166, 169-171, 173, 174, 197
Prion	134-140, 174
Production quantities	85, 86, 88, 92, 95, 102, 107, 109, 112, 158, 160, 164, 168
Protozoal parasites	127, 128
Pseudomonas	
pseudomallei	91
Pseudomonas	91, 220
Psittacosis	93
Q fever	3, 148, 149, 197
Rabies virus	167, 168
Recombinant DNA technologies	4, 80
Retroviruses	81, 169, 171, 173
Rickettsia	149, 150
akari	149
australis	149
conorii	149
coxiella burnetii	13, 148, 149
moose ri	149
Orientia	149
prowazekii	149
rickettsii	149-151
siberica	149
Spotted Fever Group	149
Surveillance of personnel	151
tsutsugamushi	149
typhi	108, 109, 149
Rickettsial agents	148, 150, 151
Rickettsial pox	150
Riderspest virus	220
Rift Valley Fever virus	192, 196, 220
Risk Assessment	viii, 4, 8, 9, 36, 48, 60, 65, 69, 72, 76-82, 84, 87, 159, 186
Rocky Mountain spotted fever	149, 150
Safety Equipment	viii, 3, 8-11, 13, 18, 20, 24, 27, 32, 42, 52, 53, 56, 60, 65, 73, 75, 86, 87, 156, 183, 185, 189, 191, 196, 240
Salmonella	108
Salmonellosis	108, 224
typhi	108, 109, 149
SALS	54, 183, 184, 189-196

Schistosoma spp.	129, 130
Screwworm	220
Serum	22, 29, 38, 39, 52, 58, 63, 70, 73, 79, 94, 154, 157, 172, 221, 235
Sharps	18, 20, 23, 28, 30, 37, 40, 52, 55, 58, 63, 71, 75, 143, 171, 203
Shigella spp.	109
Shigellosis	2, 109
Shipment	16, 215, 218, 221, 227
Simian Immunodeficiency Virus (SIV)	169
Skinhoj	2
Smallpox	3, 166, 167
Sporothrix schenckii	121
Spotted fever group	149
Strongyloides spp.	131
Suit laboratory	42, 46, 69, 73, 155, 160
Sulkin and Pike	1
Surveillance	3, 24, 32, 39, 41, 52, 55, 58, 60, 62, 63, 65, 70, 73, 75, 79, 80, 106, 150, 151, 169, 173, 237, 238
Swine vesicular disease	220
Syphilis	110
T-lymphotrophic virus	173
Taenia solium	130
Tanapox	166
Teschen disease virus	220
Tetanus	1, 84, 95, 96
Theileria	220
annulata	220
bovis	103, 104, 106, 220
hirci	220
lawrencei	220
Toxins of biological origin,	237
Toxoids	84
Toxoplasma spp.	12, 128
Trachoma	93
transfer of infectious microorganisms	ix
Transmissible spongiform encephalopathies	134, 173, 174
Transportation and Transfer of Biological Agents	214
Trematode parasites	
Fasciola	129
Schistosoma	129, 130
Treponema pallidum	110

Trichophyton	122
Trypanosoma	
cruzi	127, 220
evansi	220
vivax	127, 220
Tuberculosis	1, 2, 4, 13, 15, 84, 103-106, 223, 234
Tularemia	1, 98, 212
Typhoid fever	108
Typhus	149, 151
Universal precautions	4, 15, 77, 82, 170, 234
Vaccines ...	8, 77, 84, 85, 90-94, 97, 99-104, 107-111, 157, 158, 161, 164, 165, 167, 175, 176, 185, 189, 192, 212
Vaccinia	81, 166, 167
Variola	167
Venezuelan equine encephalomyelitis	189-191
Vesicular exanthema virus	220
Vesicular stomatitis virus	175
Vibrio	111
cholerae	111
parahaemolyticus	111
Vibrionic enteritis	111
Viral agents	153, 161, 162, 234
Viral hemorrhagic disease	220
Warning sign	29, 55, 59, 64, 71
Wesselsbron disease virus	220
Yaba	166
Yellow fever	189-192, 195, 212
Yersinia pestis	111