HHS Publication No. (CDC) 84-8395

Biosafety in Microbiological and Biomedical Laboratories

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

and

National Institutes of Health

1st Edition
March 1984

U.S. GOVERNMENT PRINTING OFFICE
WASHINGTON : 1984

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402

Editors

John H. Richardson, D.V.M., M.P.H.
Formerly Director, Office of Biosafety
Centers for Disease Control
Atlanta, Georgia

W. Emmett Barkley, Ph.D.
Director, Division of Safety
National Institutes of Health
Bethesda, Maryland

CONTRIBUTORS

CDC-NIH Interagency Working Group

Centers for Disease Control

Albert Balows, Ph.D.	Assistant Director for Laboratory Science Center for Infectious Diseases
Walter R. Dowdle, Ph.D.	Director, Center for Infectious Diseases
Karl M. Johnson, M.D.	Program Director, Hazardous Viruses U.S. Army Medical Research Institute for Infectious Diseases Formerly Chief, Special Pathogens Branch Virology Division, Bureau of Laboratories
John H. Richardson, D.V.M., M.P.H.	Formerly Director, Office of Biosafety

National Institutes of Health

W. Emmett Barkley, Ph.D.	Director, Division of Safety
John E. Bennett, M.D.	Head, Clinical Mycology Section National Institute of Allergy and Infectious Diseases
Robert Chanock, M.D.	Chief, Laboratory of Infectious Diseases National Institute of Allergy and Infectious Diseases
Franklin A. Neva, M.D.	Chief, Laboratory of Parasitic Diseases National Institute of Allergy and Infectious Diseases
Rudolf Wanner, M.D.	Retired Formerly Deputy Director Division of Safety

Guest Editors

Charles W. Beard, D.V.M., Ph.D.	Laboratory Director Southeast Poultry Research Laboratory U.S. Department of Agriculture Athens, Georgia
Kenneth I. Berns, M.D., Ph.D.	Professor and Chairman, Department of Immunology and Medical Microbiology College of Medicine, University of Florida Gainesville, Florida

Purnell W. Choppin, M.D.	Leon Hess Professor The Rockefeller University New York, New York
Richard W. Emmons, M.D., Ph.D.	Chief, Viral and Rickettsial Disease Laboratory California Department of Health Berkeley, California
William J. Hausler, Jr., Ph.D.	Director, University Hygienic Laboratory University of Iowa Iowa City, Iowa
Brinton M. Miller, Ph.D.	Formerly Department of Animal Infections Merck Sharp and Dohme Research Laboratories Rahway, New Jersey
William F. Scherer, M.D.	Deceased 1982 Formerly Chairman and Professor, Department of Microbiology Medical College, Cornell University New York, New York
Franklin H. Top, Jr., M.D.,	Director Walter Reed Army Institute of Research Washington, D.C.
Paul F. Wehrle, M.D.	Director of Pediatrics Department of Health Services, Los Angeles County— University of Southern California Medical Center Los Angeles, California

Panel Chairmen

Barnett L. Cline, M.D., Ph.D.	Professor and Chairman Department of Tropical Medicine School of Public Health and Tropical Medicine Tulane University New Orleans, Louisiana
R. Gordon Douglas, Jr., M.D.	Professor and Chairman Department of Medicine Medical College, Cornell University New York, New York
Philip Russell, M.D.	Commander Fitzsimmons Army Medical Center Aurora, Colorado

John C. Sherris. M.,D.	Professor Department of Microbiology and Immunology School of Medicine, University of Washington School of Medicine Seattle, Washington
Frank E. Swatek, Ph.D.	Professor and Chairman Department of Microbiology California State University Long Beach, California
Richard C. Tilton, Ph.D.	Professor of Laboratory Medicine Department of Laboratory Medicine School of Medicine, University of Connecticut Farmington, Connecticut
Charles L. Wissemann, Jr., M.D.	Professor and Head Department of Microbiology School of Medicine University of Maryland Baltimore, Maryland

With Special Contributions from:

Roy W. Chamberlain, Sc.D.	Retired Formerly Acting Director Virology Division Bureau of Laboratories, CDC
George P. Kubica, Ph.D.	Tuberculosis Consultant Laboratory Program Office, CDC
Frederick A. Murphy, D.V.M., Ph.D.	Director, Division of Viral Diseases Center for Infectious Diseases, CDC Formerly Professor of Microbiology College of Veterinary Medicine Colorado State University Fort Collins, Colorado
Thomas P. Monath, M.D.	Director, Division of Vector-Borne Viral Diseases Center for Infectious Diseases, CDC
James W. Smith, M.D.	Professor of Pathology Director of Clinical Microbiology Indiana University Medical Center Indianapolis, Indiana
Ellen J. Garner	Formerly Secretary Office of Biosafety, CDC

Preamble

This publication describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute biosafety levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. These recommendations are advisory and are intended to provide a voluntary guide or code of practice as well as a goal for upgrading operations. Furthermore, the recommendations are offered as a guide and reference in the construction of new laboratory facilities and in the renovation of existing facilities.

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

Contents

Section I
Introduction

Microbiology laboratories are special, often unique, work environments that may pose special infectious disease risks to persons in or near them. Personnel have contracted infections in the laboratory throughout the history of microbiology. Published reports around the turn of the century described laboratory-associated cases of typhoid, cholera, glanders, brucellosis, and tetanus.[123] In 1941, Meyer and Eddie[75] 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 Pike[113] published the first in a series of surveys of laboratory-associated infections summarizing 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 Pike[114] published the second of a series of summaries of laboratory-associated infections 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,[93] adding 641 new or previously unreported cases, and again in 1976,[90] summarizing a cumulative total of 3,921 cases. Brucellosis, typhoid, tularemia, tuberculosis, hepatitis, and Venezuelan equine encephalitis were the most commonly reported. 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 al.[53] 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, Skinhoj[104] 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 Shannon[55] indicated that medical laboratory workers in England had "a five times increased risk of acquiring tuberculosis compared with the general population." Hepatitis and shigellosis were also shown to be continuing occupational risks and together with tuberculosis were the three most commonly reported occupation-associated infections in Britain.

Although these reports suggest that laboratory personnel are 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 Shannon[55] and of Skinhoj[104] indicate that laboratory personnel have higher rates of tuberculosis, shigellosis, and hepatitis than 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 Center for Disease Control in 1947-1973,[97] no secondary cases were reported in family members or community contacts. The National Animal Disease Center has reported a similar experience,[115] with no secondary cases occurring in laboratory and nonlaboratory contacts of 18 laboratory-associated cases occurring in 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.[70] Three secondary cases of smallpox were reported in two laboratory-associated outbreaks in England in 1973[96] and 1978.[130] There were earlier reports of six cases of Q fever in employees of a commercial laundry which handled linens and uniforms from a laboratory where work with the agent was conducted,[84] one case of Q fever in a visitor to a laboratory[83], and two cases of Q fever in household contacts of a rickettsiologist.[5] These cases are representative of the sporadic nature and infrequent association of community infections with laboratories working with infectious agents.

In his 1979 review[92] Pike concluded "the knowledge, the techniques, and the equipment to prevent most laboratory infections are available." No single code of practice, standards, guidelines, or other publication, however, provides detailed descriptions of techniques, equipment, and other considerations or recommendations for the broad scope of laboratory activities conducted in the United States with a variety of indigenous and exotic infectious agents. The booklet, *Classification of Etiologic Agents on the Basis of Hazard*,[15] has, since 1969, served as a general reference for some laboratory activities utilizing infectious agents. That booklet and the concept of categorizing infectious agents and laboratory activities into four classes or levels served as a basic format for *Biosafety in Microbiological and Biomedical Laboratories*. This publication will provide specific descriptions of combinations of microbiological practices, laboratory facilities, and

safety equipment and recommendations for use in four categories or biosafety levels of laboratory operation with selected infectious agents of man.

The descriptions of biosafety levels 1-4 parallel those of P1-4 in the *NIH Guidelines for Research Involving Recombinant DNA*.[43] and are consistent with the general criteria used in assigning agents to Classes 1-4 in *Classification of Etiologic Agents on the Basis of Hazard*.[15] 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 function or activity.

Section II
Principles of Biosafety

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by 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. The purpose of containment is to reduce exposure of laboratory workers and other persons to, and to prevent escape into the outside environment of potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

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 infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required for safely handling such material. The director or person in charge of the laboratory is responsible for providing or arranging for appropriate training of personnel.

When standard laboratory practices are not sufficient to control the hazard 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 hazard associated with the agent or procedure.

Each laboratory should develop or adopt a biosafety or operations manual which identifies the hazards that will or may be encountered and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must direct laboratory activities.

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 and a variety of enclosed containers. The biological safety cabinet is the principal device used to provide containment of infectious aerosols generated by many microbiological procedures. Three types

of biological safety cabinets (Class I, II, III) used in microbiological laboratories are illustrated in Figures 1-3 and described in Appendix A. Open-fronted Class I and Class II biological safety cabinets are partial containment cabinets which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

An example of an enclosed container is the safety centrifuge cup, which is designed to prevent aerosols from being released during centrifugation.

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

Facility Design (Secondary Barriers). The design of the facility is important in providing a barrier to protect persons working in the facility but outside the laboratory and those 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. Three facility designs are described below, in ascending order by level of containment.

1. *The Basic Laboratory.* This laboratory provides general space in which work is done with viable agents which are not associated with disease in healthy adults. Basic laboratories include those facilities described in the following pages as Biosafety Levels 1 and 2 facilities.
   This laboratory is also appropriate for work with infectious agents or potentially infectious materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice. While work is commonly conducted on the open bench, certain operations are confined to biological safety cabinets. Conventional laboratory designs are adequate. Areas known to be sources of general contamination, such as animal rooms and waste staging areas, should not be adjacent to patient care activities. Public areas and general offices to which nonlaboratory staff require frequent access should be separated from spaces which primarily support laboratory functions.
2. *The Containment Laboratory.* This laboratory has special engineering features which make it possible for laboratory workers to handle hazardous materials without endangering themselves, the

community, or the environment.The containment laboratory is described in the following pages as a Biosafety Level 3 facility. The unique features which distinguish this laboratory from the basic laboratory are the provisions for access control and a specialized ventilation system. The containment laboratory may be an entire building or a single module or complex of modules within a building. In all cases, the laboratory is separated by a controlled access zone from areas open to the public.

3. *The Maximum Containment Laboratory.* This laboratory has special engineering and containment features that allow activities involving infectious agents that are extremely hazardous to the laboratory worker or that may cause serious epidemic disease to be conducted safely. The maximum containment laboratory is described on the following pages as a Biosafety Level 4 facility. Although the maximum containment laboratory is generally a separate building, it can be constructed as an isolated area within a building. The laboratory's distinguishing characteristic is that it has secondary barriers to prevent hazardous materials from escaping into the environment. Such barriers include sealed openings into the laboratory, airlocks or liquid disinfectant barriers, a clothing-change and shower room contiguous to the laboratory, a double door autoclave, a biowaste treatment system, a separate ventilation system, and a treatment system to decontaminate exhaust air.

Biosafety Levels. Four biosafety levels are described which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazard posed by the infectious agents and for the laboratory function or activity.

Biosafety Level 1 practices, safety equipment, and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis, Naegleria gruberi,* and infectious canine hepatitis virus are representative of those 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 in immunodeficient or immunosuppressed individuals. Vaccine strains which have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 2 practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents 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 aerosols is low. Hepatitis B virus, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. Primary hazards to personnel working with these agents may include accidental autoinoculation, ingestion, and skin or mucous membrane exposure to infectious materials. Procedures with high aerosol potential that may increase the risk of exposure of personnel must be conducted in primary containment equipment or devices.

Biosafety Level 3 practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences. Autoinoculation and ingestion also represent primary hazards to personnel working with these agents. Examples of such agents for which Biosafety Level 3 safeguards are generally recommended include *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii*.

Biosafety Level 4 practices, safety equipment, and facilities are applicable to work with dangerous and exotic agents which pose a high individual risk of life-threatening disease. 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. Lassa fever virus is representative of the microorganisms assigned to Level 4.

Four biosafety levels are also described for activities involving infectious disease activities with experimental mammals. 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.

The laboratory director is directly 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.

Work with known agents should be conducted at the biosafety level recommended in Section V unless specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, and other factors are significantly altered to require more stringent or allow less stringent practices to be used.

Clinical laboratories, and especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, clinical laboratories receive specimens without pertinent information such as patient history or clinical findings which may be suggestive of an infectious etiology. Furthermore, such specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputum samples 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 identification of isolates can be and are safely conducted using a combination of practices, facilities, and safety equipment described as Biosafety Level 2. Biological safety cabinets (Class I or II) should be used for the initial processing of clinical specimens when the nature of the test requested or other information is suggestive that an agent readily transmissible by infectious aerosols is likely to be present. Class II biological safety cabinets are also used to protect the integrity of the specimens or cultures by preventing contamination from the laboratory environment.

Segregating clinical laboratory functions and limiting or restricting access to laboratory areas are the responsibility of the laboratory director.

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 D).

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 E).

TABLE 1. Summary of recommended biosafety levels for infectious agents.

Biosafety Level	Practices and Techniques	Safety Equipment	Facilities
1	Standard microbiological practices	None: primary containment provided by adherence to standard laboratory practices during open bench operations.	Basic
2	Level 1 practices plus: Laboratory coats; decontamination of all infectious wastes; limited access; protective gloves and biohazard warning signs as indicated.	Partial containment equipment (i.e., Class I or II Biological Safety Cabinets) used to conduct mechanical and manipulative procedures that have high aerosol potential that may increase the risk of exposure to personnel.	Basic
3	Level 2 practices plus: special laboratory clothing; controlled access.	Partial containment equipment used for all manipulations of infectious material.	Containment
4	Level 3 practices plus: entrance through change room where street clothing is removed and laboratory clothing is put on; shower on exit; all wastes are decontaminated on exit from the facility.	Maximum containment equipment (i.e., Class III biological safety cabinet or partial containment equipment in combination with full-body, air-supplied, positive-pressure personnel suit) used for all procedures and activities.	Maximum Containment

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 and 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1 is suitable for work involving agents of no known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or 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 are in progress.
   2. Work surfaces are decontaminated once a day and after any spill of viable material.
   3. All contaminated liquid or solid wastes are decontaminated before disposal.
   4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
   5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators should be located outside of the work area.
   6. Persons wash their hands after they handle viable materials and animals and before leaving the laboratory.
   7. All procedures are performed carefully to minimize the creation of aerosols.
   8. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

B. *Special Practices*
   1. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.
   2. An insect and rodent control program is in effect.

C. *Containment Equipment*

Special containment equipment is generally not required for manipulations of agents assigned to Biosafety Level 1.

D. *Laboratory Facilities*

1. The laboratory is designed so that it can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for handwashing.
5. If the laboratory has windows that open, they are fitted with fly screens.

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs 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, and (3) certain procedures in which infectious aerosols are 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 by the laboratory director when work with infectious agents is in progress.
2. Work surfaces are decontaminated at least once a day and after any spill of viable material.
3. All infectious liquid or solid wastes are decontaminated before disposal.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators should be located outside of the work area.
6. Persons wash their hands after handling infectious materials and animals and when they leave the laboratory.
7. All procedures are performed carefully to minimize the creation of aerosols.

B. *Special Practices*

1. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.
2. The laboratory director limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.
4. When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.
5. An insect and rodent control program is in effect.
6. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for nonlaboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.
7. Animals not involved in the work being performed are not permitted in the laboratory.
8. Special care is taken to avoid skin contamination with infectious materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
9. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
10. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced

in the sheath or guard or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

11. Spills and accidents which 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. 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.
13. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

C. *Containment Equipment*

Biological safety cabinets (Class I or II) (see Appendix A) or other appropriate personal protective or physical containment devices are used whenever:

1. Procedures with a high potential for creating infectious aerosols are conducted.[82] 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 eggs.
2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

D. *Laboratory Facilities*

1. The laboratory is designed so that it can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for handwashing.
5. If the laboratory has windows that open, they are fitted with fly screens.
6. An autoclave for decontaminating infectious laboratory wastes is available.

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 material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, 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 "Containment 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. Work surfaces are decontaminated at least once a day and after any spill of viable material.
   2. All infectious liquid or solid wastes are decontaminated before disposal.
   3. Mechanical pipetting devices are used; mouth pipetting is prohibited.
   4. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.
   5. Persons wash their hands after handling infectious materials and animals and when they leave the laboratory.
   6. All procedures are performed carefully to minimize the creation of aerosols.

B. *Special Practices*
   1. Laboratory doors are kept closed when experiments are in progress.
   2. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

3. 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 be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
4. 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.
5. 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.
6. All activities 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.
7. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with infectious materials is finished. Plastic-backed paper toweling used on nonperforated work surfaces within biological safety cabinets facilitates clean-up.
8. An insect and rodent control program is in effect.
9. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.
10. Special care is taken to avoid skin contamination with infectious materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
11. Molded surgical masks or respirators are worn in rooms containing infected animals.
12. Animals and plants not related to the work being conducted are not permitted in the laboratory.
13. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

14. Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps.
15. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the sheath or guard or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
16. Spills and accidents which 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.
17. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.
18. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

C. *Containment Equipment*

Biological safety cabinets (Class I, II, or III) (see Appendix A) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with infectious materials which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs, and necropsy of infected animals.

D. *Laboratory Facilities*

1. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may also be provided

by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

2. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Each laboratory contains a sink for handwashing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
6. Windows in the laboratory are closed and sealed.
7. Access doors to the laboratory or containment module are self-closing.
8. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.
9. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room can be discharged to the outside without being filtered or otherwise treated.
10. The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection[80]) that avoids any interference with the air balance of the cabinets or building exhaust system.

Biosafety Level 4 is required for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease. 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 I or Class II biological safety cabinets used along with one-piece positive pressure personnel suits ventilated by a life support system. The maximum containment laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

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

A. *Standard Microbiological Practices*
   1. Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.
   2. Only mechanical pipetting devices are used.
   3. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.
   4. All procedures are performed carefully to minimize the creation of aerosols.

B. *Special Practices*
   1. Biological materials to be removed from the Class III cabinet or from the maximum containment laboratory in a viable or intact state are transferred to a nonbreakable, sealed primary container and then enclosed in a nonbreakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.
   2. No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before they leave the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

3. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous 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, biohazards control 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 insuring 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.
4. Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Personnel use the airlocks to enter or leave the laboratory only in an emergency.
5. Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including under garments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room.
6. When infectious materials or infected animals are present in the laboratory or animal rooms, a hazard warning sign, incorporating the universal biohazard symbol, is 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).
7. 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.
8. An insect and rodent control program is in effect.

9. Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.
10. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of infectious fluids. Needles should not be bent, sheared, replaced in the needle guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).
11. A system is set up 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.

C. *Containment Equipment*

1. All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system. Activities with viral agents (e.g., Rift Valley fever virus) that require Biosafety Level 4 secondary containment capabilities and for which highly effective vaccines are available and used can be conducted within Class I or Class II biological safety cabinets within the facility without the one-piece positive pressure personnel suit being used if (1) the facility has been decontaminated, (2) no work is being conducted in the facility with other agents assigned to Biosafety Level 4, and (3) all other standard and special practices are followed.

D. *Laboratory Facility*

1. The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

2. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain HEPA filters.
3. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.
4. Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
5. Laboratory furniture is of simple and sturdy construction, and spaces between benches, cabinets, and equipment are accessible for cleaning.
6. A foot, elbow, or automatically operated handwashing sink is provided near the door of each laboratory room in the facility.
7. If there is a central vacuum system, it does not serve areas outside the facility. 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 facility are protected by devices that prevent backflow.
8. If water fountains are provided, they are foot operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the backflow-protected distribution system supplying water to the laboratory areas.
9. Access doors to the laboratory are self-closing and lockable.
10. Any windows are breakage resistant.
11. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.
12. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.
13. Liquid effluents from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated

by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower rooms are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

14. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.
15. The exhaust air from the facility is filtered through HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.
16. The treated exhaust air from Class I and II biological safety cabinets can be discharged into the laboratory room environment or to the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at 6-month intervals. *The treated exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of HEPA filters in series, via the facility exhaust air system.* If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection[80]) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

17. A specially designed suit area may be provided in the facility.Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life support system. The life support system includes 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. The exhaust air from the suit area is filtered by two sets of HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. 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 are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit area.

TABLE 2. Summary of recommended biosafety levels for activities in which experimentally or naturally infected vertebrate animals are used.

Biosafety Level	Practices and Techniques	Safety Equipment	Facilities
1	Standard animal care and management practices.	None	Basic
2	Laboratory coats; decontamination of all infectious wastes and of animal cages prior to washing; limited access; protective gloves and hazard warning signs as indicated.	Partial containment equipment and/or personal protective devices used for activities and manipulations of agents or infected animals that produce aerosols.	Basic
3	Level 2 practices plus: special laboratory clothing; controlled access.	Partial containment equipment and/or personal protective devices used for all activities and manipulations of agents or infected animals.	Containment
4	Level 3 practices plus: entrance through clothes change room where street clothing is removed and laboratory clothing is put on shower on exit; all wastes are decontaminated before removal from the facility.	Maximum containment equipment (i.e., Class III biological safety cabinet or partial containment equipment in combination with full-body, air-supplied positive-pressure personnel suit) used for all procedures and activities.	Maximum Containment

Section IV
Vertebrate Animal Biosafety Level Criteria

If experimental animals are used, institutional management must provide facilities and staff and establish practices which reasonably assure appropriate levels of environmental quality, safety, and care. Laboratory animal facilities are extensions of the laboratory and in some situations are integral to and inseparable from the 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.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations and that appropriate species have been selected for animal experiments (e.g., *Guide for the Care and Use of Laboratory Animals*, HEW Publication No. (NIH) 78-23, Rev. 1978, and *Laboratory Animal Welfare Regulations* - 9 CFR, Subchapter A, Parts 1, 2 and 3).

Ideally, facilities for laboratory animals used for 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 that provide patient care. Animal facilities should be designed and constructed to facilitate cleaning and housekeeping. A "clean hall/dirty hall" layout is very useful in reducing cross contamination. Floor drains should be installed in animal facilities only on the basis of clearly defined needs. If floor drains are installed, the drain trap should always contain water.

These recommendations describe four combinations of practices, safety equipment, and facilities for experiments on animals infected with agents which are known or believed to produce infections in humans. These four combinations provide increasing levels of protection to personnel and to the environment and are recommended as minimal standards for activities involving infected laboratory animals. These four combinations, designated Animal Biosafety Levels 1-4, describe animal facilities and practices applicable to work on animals infected with agents assigned to corresponding Biosafety Levels 1-4.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in standards written for commonly used laboratory animals. "Laboratory Safety for Arboviruses and Certain other Viruses of Vertebrates,"[112] prepared by the Subcommittee on Arbovirus Laboratory Safety 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

A. *Standard Practices*

1. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present.
2. Work surfaces are decontaminated after use or after any spill of viable materials.
3. Eating, drinking, smoking, and storing food for human use are not permitted in animal rooms.
4. Personnel wash their hands after handling cultures and animals and before leaving the animal room.
5. All procedures are carefully performed to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. *Special Practices*

1. Bedding materials from animal cages are removed in such a manner as to minimize the creation of aerosols and disposed of in compliance with applicable institutional or local requirements.
2. Cages are washed manually or in a cagewasher. Temperature of final rinse water in a mechanical washer should be 180°F.
3. The wearing of laboratory coats, gowns, or uniforms in the animal room is recommended. It is further recommended that laboratory coats worn in the animal room not be worn in other areas.

C. *Containment Equipment*

Special containment equipment is not required for animals infected with agents assigned to Biosafety Level 1.

D. *Animal Facilities*

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A handwashing sink is available in the animal facility.
3. If the animal facility has windows that open, they are fitted with fly screens.
4. It is recommended, but not required, that the direction of airflow in the animal facility is inward and that exhaust air is discharged to the outside without being recirculated to other rooms.

Animal Biosafety Level 2

A. *Standard Practices*
   1. Doors to animal rooms open inward, are self-closing, and are kept closed when infected animals are present.
   2. Work surfaces are decontaminated after use or spills of viable materials.
   3. Eating, drinking, smoking, and storing of food for human use are not permitted in animal rooms.
   4. Personnel wash their hands after handling cultures and animals and before leaving the animal room.
   5. All procedures are carefully performed to minimize the creation of aerosols.
   6. An insect and rodent control program is in effect.

B. *Special Practices*
   1. Cages are decontaminated, preferably by autoclaving, before they are cleaned and washed.
   2. Surgical-type masks are worn by all personnel entering animal rooms housing nonhuman primates.
   3. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.
   4. The laboratory or animal facility director limits access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. 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 room.
   5. The laboratory or animal facility director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., for immunization) may enter the animal room.
   6. When the infectious agent(s) in use in the animal room requires special entry provisions (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the animal room. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the animal facility supervisor or other responsible person(s), and indicates the special requirement(s) for entering the animal room.
   7. Special care is taken to avoid skin contamination with infectious materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
   8. All wastes from the animal room are appropriately decontaminated—preferably by autoclaving—before disposal.

Infected animal carcasses are incinerated after being transported from the animal room in leakproof, covered containers.

9. Hypodermic needles and syringes are used only for the parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable needle syringe units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
11. When appropriate, considering the agents handled, baseline serum samples from animal care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically, depending on the agents handled or the function of the facility.

C. *Containment Equipment*

1. Biological safety cabinets, other physical containment devices, and/or personal protective devices (e.g., respirators, face shields) are used whenever procedures with a high potential for creating aerosols are conducted.[82] These include necropsy of infected animals, harvesting of infected tissues or fluids from animals or eggs, intranasal inoculation of animals, and manipulations of high concentrations or large volumes of infectious materials.

D. *Animal Facilities*

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A handwashing sink is available in the room where infected animals are housed.
3. If the animal facility has windows that open, they are fitted with fly screens.
4. It is recommended, but not required, that the direction of airflow in the animal facility is inward and that exhaust air is discharged to the outside without being recirculated to other rooms.
5. An autoclave which can be used for decontaminating infectious laboratory waste is available in the building with the animal facility.

Animal Biosafety Level 3

A. *Standard Practices*

1. Doors to animal rooms open inward, are self-closing and are kept closed when work with infected animals is in progress.
2. Work surfaces are decontaminated after use or spills of viable materials.
3. Eating, drinking, smoking, and storing of food for human use are not permitted in the animal room.
4. Personnel wash their hands after handling cultures and animals and before leaving the laboratory.
5. All procedures are carefully performed to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. *Special Practices*

1. Cages are autoclaved before bedding is removed and before they are cleaned and washed.
2. Surgical-type masks or other respiratory protection devices (e.g., respirators) are worn by personnel entering rooms housing animals infected with agents assigned to Biosafety Level 3.
3. Wrap-around or solid-front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective gowns must remain in the animal room and must be decontaminated before being laundered.
4. The laboratory director or other responsible person restricts access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when infected animals are present. 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 room.
5. The laboratory director or other responsible person establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., for immunization) may enter the animal room.
6. Hazard warning signs, incorporating the universal biohazard warning symbol, are posted on access doors to animal rooms containing animals infected with agents assigned to Biosafety Level 3 are present. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the animal room supervisor or other responsible person(s), and indicate any special conditions of entry into the animal room (e.g., the need for immunizations or respirators).

7. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before being disposed of or reused.
8. All wastes from the animal room are autoclaved before disposal. All animal carcasses are incinerated. Dead animals are transported from the animal room to the incinerator in leak-proof covered containers.
9. Hypodermic needles and syringes are used only for gavage or for parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable needle syringe units (i.e., the needle is integral to the syringe) are used. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferable by autoclaving, before discard or reuse. Whenever possible, cannulas should be used instead of sharp needles (e.g., gavage).
10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
11. If vacuum lines are provided, they are protected with HEPA filters and liquid disinfectant traps.
12. Boots, shoe covers, or other protective footwear and disinfectant footbaths are available and used when indicated.

C. *Containment Equipment*

1. Personal protective clothing and equipment and/or other physical containment devices are used for all procedures and manipulations of infectious materials or infected animals.
2. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in partial containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar flow cabinets) solid wall and bottom cages covered by filter bonnets, or other equivalent primary containment systems.

D. *Animal Facilities*

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping and is separated from areas which are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or other activities may also be provided by a double-doored clothes change room (showers may be

included), airlock, or other access facility which requires passage through two sets of doors before entering the animal room.

2. The interior surfaces of walls, floors, and ceilings are water resistant so that they may be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate fumigation or space decontamination.
3. A foot, elbow, or automatically operated handwashing sink is provided near each animal room exit door.
4. Windows in the animal room are closed and sealed.
5. Animal room doors are self-closing and are kept closed when infected animals are present.
6. An autoclave for decontaminating wastes is available, preferably within the animal room. Materials to be autoclaved outside the animal room are transported in a covered leakproof container.
7. An exhaust air ventilation system is provided. This system creates directional airflow that draws air into the animal room through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the animal room) is proper. The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.
8. The HEPA filtered exhaust air from Class I or Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble unit connection[80]) that avoids any interference with the air balance of the cabinets or building exhaust system.

Animal Biosafety Level 4

A. *Standard Practices*

1. Doors to animal rooms open inward and are self-closing.
2. Work surfaces are decontaminated after use or spills of viable materials.
3. Eating, drinking, smoking, and storing of food for human use is not permitted in the animal room.
4. All procedures are carefully performed to minimize the creation of aerosols.
5. An insect and rodent control program is in effect.
6. Cages are autoclaved before bedding is removed and before they are cleaned and washed.

B. *Special Practices*

1. Only persons whose entry into the facility or individual animal rooms is required for program or support purposes are authorized to enter. 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. Persons at increased risk may include children, pregnant women, and persons who are immunodeficient or immunosuppressed. 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 secure, locked doors; accessibility is controlled by the animal facility supervisor, biohazards control 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. Personnel comply with the instructions and all other applicable entry and exit procedures. Practical and effective protocols for emergency situations are established.
2. Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Head covers are provided to personnel who do not wash their hair during the exit shower. Except in an emergency, personnel do not enter or leave the facility through the airlocks.
3. Street 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, are provided and used by all personnel entering the facility. When exiting, personnel remove laboratory clothing and store it in a locker or hamper in the inner change room before entering the shower area.
4. When infectious materials or infected animals are present in the animal rooms, a hazard warning sign, incorporating the uni-

versal biohazard symbol, is posted on all access doors. The sign identifies the agent, lists the name and telephone number of the animal facility supervisor or other responsible person(s), and indicates any special conditions of entry into the area (e.g., the need for immunizations and respirators).

5. Supplies and materials to be taken into the facility enter by way of the double-door autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. After securing the outer doors, personnel inside the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. This inner door is secured after materials are brought into the facility.
6. Materials (e.g., plants, animals, clothing) not related to the experiment are not permitted in the facility.
7. Hypodermic needles and syringes are used only for gavage or for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used. Needles should not be bent, sheared, replaced in the guard or sheath, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse. Whenever possible, cannulas should be used instead of sharp needles (e.g., gavage).
8. A system is developed and is operational for the reporting of animal facility accidents and exposures, 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.
9. Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.

C. *Containment Equipment*

Laboratory animals, infected with agents assigned to Biosafety Level 4, are housed in the Class III biological safety cabinet or in partial containment caging systems (such as open cages placed in ventilated enclosures, solid wall and bottom cages covered with filter bonnets, or other equivalent primary containment systems) in specially designed areas in which all personnel are required to wear one-piece positive pressure suits ventilated with a life support system. Animal work with viral

agents that require Biosafety Level 4 secondary containment and for which highly effective vaccines are available and used may be conducted with partial containment cages and without the one-piece positive pressure personnel suit if the facility has been decontaminated, if no concurrent experiments are being done in the facility which require Biosafety Level 4 primary and secondary containment, and if all other standard and special practices are followed.

D. *Animal Facility*

1. The animal rooms are located in a separate building or in a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of materials, supplies, or equipment which are not brought into the facility through the change room.
2. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed.
3. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.
4. A foot, elbow, or automatically operated handwashing sink is provided near the door of each animal room within the facility.
5. If there is a central vacuum system, it does not serve areas outside of the facility. The vacuum system has in-line HEPA filters 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 for the facility are protected by devices that prevent backflow.
6. External animal facility doors are self-closing and self-locking.
7. Any windows must be resistant to breakage and sealed.
8. A double-doored autoclave is provided for decontaminating materials that leave the facility. The autoclave door which opens to the area external to the facility is automatically controlled so that it can only be opened after the autoclave "sterilization" cycle is completed.
9. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.
10. Liquid effluents from laboratory sinks, cabinets, floors, and autoclave chambers are decontaminated by heat treatment

before being discharged. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes must be evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower rooms are decontaminated with chemical disinfectants, the chemicals used must have documented efficacy against the target or indicator microorganisms.

11. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials, and directional airflow is required to assure inflow from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are provided to sense pressure differentials between adjacent areas that are maintained at different pressure levels. The manometers sound an alarm when a system malfunctions. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.
12. Air can be recirculated within an animal room if it is filtered through a HEPA filter.
13. The exhaust air from the facility is filtered by HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near to the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters are provided for treatment of air supplied to the facility in order to increase the lifetime of the HEPA filters.
14. The treated exhaust air from Class I or Class II biological safety cabinets can be discharged into the animal room environment or to the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the animal room the cabinets are tested and certified at 6-month intervals. *The treated exhaust air from Class III biological safety cabinets is discharged without recirculation via the facility exhaust air system.* If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air 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 air system.
15. A specially designed suit area may be provided in the facility. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life support system. The life sup-

port system is provided with 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. The exhaust air from the area in which the suit is used is filtered by two sets of HEPA filters installed in series. A duplicate filtration unit and exhaust fan are provided. An automatically starting emergency power source is provided. 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 inner shell of the suit area are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit area.

Section V
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. 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 quantity and concentration of 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, tubercle bacilli); the potential for laboratory-associated infection is high even in the absence of previously documented laboratory-associated infections (e.g., exotic arboviruses); or, the consequences of infection are grave (e.g., Creutzfeldt-Jakob disease, botulism).

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. 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 attenuated virus vaccines in individuals with altered immunocompetence.

Risk assessments and Biosafety Levels recommended in the agent summary statements presuppose a population of immunocompetent individuals. Those with altered immunocompetence may be at increased risk when exposed to infectious agents. Immunodeficiency may be hereditary, congenital, or induced by a number of neoplastic diseases, by therapy, or by radiation. The risk of becoming infected or the consequences 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 individualizing the generic risk assessments of the agent summary statements for specific activities.

The basic biosafety level assigned to an agent is based on the activities typically associated with the growth and manipulation of quantities and concentrations of infectious agents required to accomplish identification or typing. If activities with clinical materials pose a lower 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 or highly 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 possible to define "production quantities" in finite volumes or concentrations for any given agent. Therefore, the laboratory director must make a risk 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 "Containment Equipment" for Biosafety Level 3 are rigorously followed. 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 selecting appropriate safeguards for materials that may contain a suspected agent. For example, sera of human origin may contain hepatitis B virus and should be handled under conditions which reasonably preclude cutaneous, mucous membrane or parenteral exposure of personnel; and sputa submitted to the

laboratory for assay for tubercle bacilli should be handled under conditions which reasonably preclude the generation of aerosols or which contain any aerosols that may be generated during the manipulation of clinical materials or cultures.

The infectious agents which meet the previously stated criteria are listed by category of agent on the following pages. 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 for working with clinical materials, cultures of infectious agents, or infected animals recommended in the agent summary statement and described in Section V.

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

Risk Assessment

The risk assessment of laboratory activities involving the use of infectious microorganisms is ultimately a subjective process. Those risks associated with the agent, as well as with the activity to be conducted, must be considered in the assessment. The characteristics of infectious agents and the primary laboratory hazards of working with the agent are described generically for agents in Biosafety Levels 1-4 and specifically for individual agents or groups of agents on pages 6 and 7 and in Section V, respectively, of this publication.

Hepatitis B virus (HBv) is an appropriate model for illustrating the risk assessment process. HBv is among the most ubiquitous of human pathogens and most prevalent of laboratory-associated infections. The agent has been demonstrated in a variety of body secretions and excretions. Blood, saliva, and semen have been shown to be infectious. Natural transmission is associated with parenteral inoculation or with contamination of the broken skin or of mucous membranes with infectious body fluids. There is no evidence of airborne or interpersonal spread through casual contact. Prophylactic measures include the use of a licensed vaccine in high-risk groups and the use of hepatitis B immune globulin following overt exposure.

The primary risk of HBv infection in laboratory personnel is associated with accidental parenteral inoculation, exposure of the broken skin or mucous membranes of the eyes, nose, or mouth, or ingestion of infectious body fluids. These risks are typical of those described for Biosafety Level 2 agents and are addressed by using the recommended standard and special microbiological practices to minimize or eliminate these overt exposures.

Hepatitis nonA-nonB and AIDS—acquired immune deficiency syndrome—pose similar infection risks to laboratory personnel. The prudent practices recommended for HBv are applicable to these two disease entities, as well as to the routine laboratory manipulation of clinical materials of domestic origin.

The described risk assessment process is also applicable to laboratory operations other than those involving the use of primary agents of human disease. Microbiological studies of animal host-specific pathogens, soil, water, food, feeds, and other natural or manufactured materials, by comparison, pose substantially lower risks of laboratory infection. Microbiologists and other scientists working with such materials may, nevertheless, find the practices, containment equipment, and facility recommendations described in this publication of value in developing operational standards to meet their own assessed needs.

Agent Summary Statements

• *Parasitic Agents*

Agent: **Nematode Parasites of Humans**

Laboratory-associated infections with *Strongyloides* spp.and hookworms have been reported.[90] 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 and of dogs infected with *Strongyloides* spp. are a potential infection hazard for laboratory and animal care personnel.

Laboratory Hazard: 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. 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, containment equipment, and facilities are recommended for activities with *infective stages* of the parasites 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.

Agent: **Protozoal Parasites of Humans**

Laboratory-associated infections with *Toxoplasma* spp.; *Plasmodium* spp. (including *P. cynomologi*); *Trypanosoma* spp.; and *Leishmania* spp.; have been reported.[49,21,90,100] In addition, infections with *Entamoeba histolytica, Giardia* spp., and *Coccidia* spp. can result from ingestion of cysts in feces.

Accidental laboratory infections as well as human volunteer studies have proven the transmissibility of *Plasmodium cynomologi* from nonhuman primates to humans via infected mosquitoes.[40] Although laboratory animal-associated infections have not been reported, contact with lesion material from rodents with cutaneous leishmaniasis and with feces or blood of experimentally or naturally infected animals may be a direct source of infection for laboratory personnel.

Laboratory Hazard: Infective stages may be present in blood, feces, lesion exudates, and infected arthropods. Depending on the parasite, accidental parenteral inoculation, transmission by arthropod vectors, skin penetration, and ingestion are the primary laboratory hazards. Aerosol or droplet exposure of the mucous membranes of the eyes, nose, or mouth

with trophozoites are potential hazards when working with cultures of *Naegleria fowleri, Leishmania* spp., *T. cruzi,* or with tissue homogenates or blood containing hemoflagellates. Because of the grave consequence of toxoplasmosis in the developing fetus, women of childbearing age should be discouraged from working with viable *Toxoplasma* spp.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, 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 *T. cruzi, Leishmania, Naegleria fowleri,* or with tissue homogenates or blood containing hemoflagellates. Gloves are recommended for activities where there is the likelihood of direct skin contact with infective stages of the parasites listed.

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

Laboratory-associated infections with *Schistosoma* spp. and *Fasciola* spp. have been reported—none associated directly with laboratory animals.[90]

Laboratory Hazard: Infective stages of *Schistosoma* spp. (cercariae) and *Fasciola* spp. (metacercariae) 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 metacercariae are the primary laboratory hazards. Dissection or crushing of schistosome-infected snails may also result in exposure of skin or mucous membrane to cercariae-containing droplets. Additionally, metacercariae may be inadvertently transferred from hand to mouth by fingers or gloves following contact with contaminated aquatic vegetation or 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, containment equipment, 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 metacercariae from naturally or experimentally infected snail intermediate hosts. Snails and cercariae in the water of laboratory aquaria should be killed by chemicals (e.g., hypochlorites, iodine) or heat before discharge to sewers.

Agent: **Cestode Parasites of Humans** (*Echinococcus granulosus* and *Taenia solium* (cysticercus cellulosae))

Although laboratory-associated infections with *E. granulosus* or *T. solium* have not been reported, the consequences of such infections fol-

lowing the ingestion of infective eggs of *T. solium* or *E. granulosus* are potentially grave.

Laboratory Hazard: 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 are the primary laboratory hazard. Cysts and cyst fluids of *E. granulosus* are not infectious for humans.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, 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 surfaces contaminated with fresh feces of dogs infected with *E. granulosus* or humans infected with *T. solium* adults.

• *Fungal Agents*

*Agent: **Blastomyces dermatitidis***

Laboratory-associated local infections following accidental parenteral inoculation with infected tissues or cultures containing yeast forms of *B. dermatitidis* [39,54,66,103,127] have been reported. A single pulmonary infection (asymptomatic) occurred following the presumed inhalation of conidia. Subsequently this individual developed an osteolytic lesion from which *B. dermatitidis* was cultured.[30] Presumably, pulmonary infections are associated only with sporulating mold forms (conidia).

Laboratory Hazard: 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 may pose a hazard of aerosol exposure.

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

Biosafety Level 3 practices, containment equipment, and facilities are recommended for processing mold cultures, soil, and other environmental materials known or likely to contain infectious conidia.

*Agent: **Coccidioides immitis***

Laboratory-associated coccidioidomycosis is a documented hazard.[12,28,31,32,33,64,68,79,105,106,107] 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.[128]

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

Spherules of the fungus may be present in clinical specimens and animal tissues and infectious arthrospores in mold cultures and soil samples. Inhalation of arthrospores from soil samples, mold cultures, or following transformation from the spherule form in clinical materials is the primary laboratory hazard. Accidental percutaneous inoculation of the spherule form may result in local granuloma formation.[118] Disseminated disease may occur at a greater frequency pregnant women, blacks, and Filipinos than in whites.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens and 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 all activities with sporulating mold form cultures of *C. immitis* and for processing soil or other environmental materials known or likely to contain infectious arthrospores.

Agent: ***Cryptococcus neoformans***

A single account of a laboratory exposure to *Cryptococcus neoformans* as a result of a laceration by a scalpel blade heavily contaminated with encapsulated cells is reported.[50] This vigorous exposure which did not result in local or systemic evidence of infection suggests that the level of pathogenicity for normal immunocompetent adults is low. Respiratory infections as a consequence of laboratory exposure have not been recorded.

Laboratory Hazards: Accidental parenteral inoculation of cultures or other infectious materials represents a potential hazard to laboratory personnel—particularly to those that 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, containment equipment, 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.

Agent: ***Histoplasma capsulatum***

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.[90,91] Pulmonary infections have resulted from handling mold form cultures.[78] Local infection has resulted from skin puncture during autopsy of an infected human[119] and from accidental needle inoculation of a viable culture.[116] Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers. Spores are resistant to drying and may remain viable for long periods of time. The small size of the infective conidia (microconidia are 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.[45]

Laboratory Hazard: 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, containment equipment, and facilities are recommended for handling and processing clinical specimens, animal tissues, and for experimental animal studies when the route of challenge is parenteral.

Biosafety Level 3 practices and facilities are recommended for processing mold cultures, soil, or other environmental materials known or likely to contain infectious conidia.

Agent: ***Sporothrix schenckii***

S. schenckii has caused a substantial number of local skin or eye infections in laboratory personnel. Most cases have been associated with accidents and have involved splashing culture material into the eye,[41,125] scratching[13] or injecting[117] infected material into the skin or being bitten by an experimentally infected animal.[60,61] Skin infections have resulted also from handling cultures[74,81] or necropsy of animals[44] without a known break in technique. No pulmonary infections have been reported to result from laboratory exposure, although naturally occurring lung disease, albeit rare, is thought to result from inhalation.

Recommended Precautions: Biosafety Level 2 and Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for all laboratory and experimental animal activities with *S. schenckii.*

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

Although skin, hair and nail infections by these dermatophytic 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, rarely, with handling cultures.[71,90,51].

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, containment equipment, and facilities recommended for all laboratory and experimental animal activities with dermatophytes.

• *Bacterial Agents*

*Agent: **Bacillus anthracis***

Forty (40) cases of laboratory-associated anthrax, occurring primarily at facilities conducting anthrax research, have been reported.[38,90] No laboratoryassociated cases of anthrax have been reported in the United States for more than 20 years.

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, 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 and facilities are recommended for studies utilizing experimentally infected laboratory rodents. A licensed vaccine is available through the Centers for Disease Control; however, vaccination of laboratory personnel is not recommended unless frequent work with clinical specimens or diagnostic cultures is anticipated (e.g., animal disease diagnostic laboratory). Biosafety Level 3 practices and facilities are recommended for work involving production volumes or concentrations of cultures and for activities which have a high potential for aerosol production. In these facilities vaccination 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.

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

B. abortus, B. canis, B. melitensis, and *B. suis* have all caused illness in laboratory personnel.[77,90,110] Brucellosis is the most commonly reported laboratory-associated bacterial infection.[90] 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, CSF, semen, and occasionally urine. Most laboratory-associated cases have occurred in research facilities and have involved exposure to *Brucella* organisms being grown in large quantities. Direct skin contact with cultures or with infectious clinical specimens from animals (e.g., blood, uterine discharges) are also commonly implicated. Aerosols generated during laboratory procedures have caused large outbreaks.[59] 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 materials 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. Vaccines are not available for use in humans.

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

Infections with psittacosis, lymphogranuloma venereum (LGV), and trachoma are documented hazards and the fifth most commonly reported laboratory-associated bacterial infection. The majority of cases were of psittacosis, occurred before 1955, and had the highest case fatality rate of all groups of infectious agents [90]. 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 Hazard: 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 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 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 and facilities and respiratory protection are recommended for personnel working with caged birds naturally or experimentally infected. 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. Additional primary containment and personnel precautions, such as those recommended for Biosafety Level 3 may be indicated for activities with high potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. Vaccines are not available for use in humans.

Agent: ***Clostridium botulinum***

While there are no reported cases 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 would be grave.

Laboratory Hazard: Cl. 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 *Cl. botulinum* is the primary laboratory hazard. The toxin may be absorbed after ingestion or following contact with the skin, eyes, or mucous membranes, including the respiratory tract. Accidental parenteral inoculation may also represent a significant exposure to toxin. Broth cultures grown under conditions of optimal toxin production may contain 2 X 10^6 mouse LD_{50} per mL.[111]

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities with materials known or potentially containing the toxin. A pentavalent (ABCDE) botulism toxoid is available through the Centers for Disease Control, as an Investigational New Drug (IND). This toxoid is recommended for personnel working with cultures of *Cl. botulinum* or its toxins. Solutions of 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, may be indicated for activities with a high potential for aerosol or droplet production, those involving production quantities of toxin, and those involving purified toxins. Animal Biosafety Level 2 practices and facilities are recommended for diagnostic studies and titration of toxin.

Agent: ***Clostridium tetani***

Although the risk of infection to laboratory personnel is negligible, Pike[90] has recorded 5 incidents related to exposure of personnel during manipulation of the toxin.

Laboratory Hazards: Accidental parenteral inoculation and ingestion of the toxin are the primary hazards to laboratory personnel. Since tetanus toxin is poorly absorbed through mucous membranes, aerosols and droplets probably represent minimal hazards.

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 may further reduce the risk to laboratory and animal care personnel of toxin exposures and wound contamination.[24]

Agent: ***Corynebacterium diphtheriae***

Laboratory-associated infections with *C. diphtheriae* are documented. Pike[90] lists 33 cases reported in the world literature.

Laboratory animal-associated infections have not been reported.

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 to laboratory and animal care personnel of toxin exposures and work with infectious materials.[24].

Agent: Francisella tularensis

Tularemia is the third most commonly reported laboratory-associated bacterial infection.[90] 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.

Laboratory Hazards: The agent may be present in lesion exudate, 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. Cultures have been more commonly associated with infection than clinical materials and infected animals. The human ID 25-50 is on the order of 10 organisms by the respiratory route.[121]

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 tularense*. Biosafety Level 3 and Animal Biosafety Level 3 practices and facilities are recommended, respectively, for all manipulations of cultures and for experimental animal studies. An investigational live attenuated vaccine[10] is available through the Centers for Disease Control and is recommended for persons working with the agent or with infected animals and for persons working in or entering the laboratory or animal room where cultures or infected animals are maintained.

Agent: Leptospira interrogans—all serovars

Leptospirosis is a well-documented laboratory hazard. Sixty-seven laboratory-associated infections and 10 deaths have been reported.[90]

An experimentally infected rabbit was identified as the source of an infection with *L. interrogans* serovar *icterohemorrhagiae*.[97] 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 available for use in humans.

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. pneumophila*) is recorded.[16] Human-to-human spread has not been documented.

Experimental infections are readily produced in guinea pigs and embryonated chicken eggs.[72] Challenged rabbits develop antibodies but not clinical disease. Mice are refractory to parenteral exposure. Unpublished studies by Kaufmann, Feeley and others at the Centers for Disease Control 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 fluids, tissue, sputa, and environmental sources (e.g., cooling tower water). Since 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 concentrations of infectious materials (e.g., infected yolk sacks 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. Primary containment devices and equipment (e.g., biological safety cabinets, centrifuge safety cups) should be used for activities likely to generate potentially infectious aerosols. Vaccines are not available for use in humans.

Agent: Mycobacterium leprae

Inadvertent parenteral human-to-human transmission of leprosy following an accidental needle stick in a surgeon[69] and the use of a presumably contaminated tattoo needle[87] have been reported. There are no cases

reported as a result of working in a laboratory with biopsy or other clinical materials of human or animal origin. While naturally occurring leprosy or leprosy-like diseases have been reported in armadillos[120] and in nonhuman primates,[35,76] humans are the only known important reservoir of this disease.

Laboratory Hazard: 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 and facilities are recommended for animal studies utilizing rodents, armadillos, and nonhuman primates.

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.[90] Presumably these infections were due to mycobacteria other than *M. tuberculosis* or *M. bovis.* A number of mycobacteria which 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:

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

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. Infectious aerosols created during the manipulation of broth cultures or tissue homogenates of these

organisms associated with pulmonary disease also pose a potential infection hazard to laboratory personnel.

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 and facilities are recommended for animal studies with the mycobacteria other than *M. tuberculosis, M. bovis*, or *M. leprae*.

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

Mycobacterium tuberculosis and *M. bovis* infections are a proven hazard to laboratory personnel as well as to others who may be exposed to infectious aerosols in the laboratory.[90,93] The incidence of tuberculosis in laboratory workers working with *M. tuberculosis* is three times higher than that of laboratorians not working with the agent.[95] 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 less than 3/10,000 in the general population).[62] 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 Hazard: Tubercle bacilli may be present in sputum, gastric lavage fluids, CFS fluid, urine, and in lesions from a variety of tissues.[3] Exposure to laboratory-generated aerosols is the most important hazard encountered. Tubercle bacilli may survive in heat-fixed smears[1] and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low ID of *M. tuberculosis* for humans (i.e., ID_{50} < 10 bacilli)[98,99] and in some laboratories a high rate of isolation of acid-fast organisms from clinical specimens (>10%),[47] 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, containment equipment, and facilities (See (American Thorasic Society) laboratory service levels I and II)[2,65] are recommended for preparing acid-fast smears and for culturing sputa or other clinical specimens, provided that aerosol-generating manipulations of such specimens are conducted in a Class I or II biological safety cabinet. Liquification and concentration of sputa for acid-fast staining may also be conducted on the open bench at Biosafety Level 2 by first treating the specimen with an equal volume of 5% sodium hypochlorite solution (undiluted household bleach) and waiting 15 minutes before centrifugation.[85,108]

Biosafety Level 3 practices, containment equipment, and facilities (See ATS laboratory service level III)[2,65] are recommended for activities involving 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. Skin testing with purified protein derivative (PPD) of previously skin-tested-negative laboratory personnel can be used as a surveillance procedure. A licensed attenuated live vaccine (BCG) is available but is not routinely used in laboratory personnel.

Agent: ***Neisseria gonorrhoeae***

Four cases of laboratory-associated gonorrhoea have been reported in the United States.[34,90]

Laboratory Hazards: The agent may be present in conjunctival, urethral and cervical exudates, synovial fluid, urine, feces, and CSF. Accidental parenteral inoculation and direct or indirect contact of mucous membranes with infectious clinical materials are the 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 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 available for use in humans.

Agent: ***Neisseria meningitidis***

Meningococcal meningitis is a demonstrated but rare hazard to laboratory workers.[4,92]

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

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities recommended for all activities utilizing known or potentially infectious body fluids and tissues. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3 may be indicated for activities with high potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. The use of licensed polysaccharide vaccines[19] should be considered for personnel regularly working with large volumes or high concentrations of infectious materials.

Agent: ***Pseudomonas pseudomallei***

Two laboratory-associated cases of melioidosis are reported: one associated with a massive aerosol and skin contact exposure;[48] the second re-

sulting from an aerosol created during the open-flask sonication of a culture presumed to be *Ps. cepacia*.[102]

Laboratory Hazards: The agent may be present in sputa, blood, wound exudates and various tissues depending on site of localization of the infection. Direct contact with cultures and 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 and tissues. Gloves should be worn when handling, and during necropsy of, infected 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 activities with a high potential for aerosol or droplet production and for activities involving production quantities or concentrations of infectious materials.

Agent: ***Salmonella cholerasuis, S. enteritidis*—all serotypes**

Salmonellosis is a documented hazard to laboratory personnel.[90] 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 Hazard: The agent may be present in feces, blood, and 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 known or potentially containing the agents. Animal Biosafety Level 2 practices and facilities are recommended for activities with experimentally or naturally infected animals.

Agent: ***Salmonella typhi***

Typhoid fever is a demonstrated hazard to laboratory personnel.[7,92]

Laboratory Hazards: The agent may be present in feces, blood, gallbladder (bile) and urine. Humans are the only known reservoir of infection. Ingestion or parenteral inoculation of the organism represent 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 utilizing known or potentially infectious clinical materials and cultures.

Licensed vaccines, which have been shown to protect 70-90% of recipients, may be a valuable adjunct to good safety practices in personnel regularly working with cultures or clinical materials which may contain *S. typhi*.[7]

Agent: ***Shigella*** **spp.**

Shigellosis is a demonstrated hazard to laboratory personnel, with 49 cases reported in the United States.[90] While outbreaks have occurred in captive nonhuman primates, humans are the only significant reservoir of infection. Experimentally infected guinea pigs, other rodents, and nonhuman primates are a proven source of infection.

Laboratory Hazards: The agent may be present in feces and, rarely, in blood of infected humans or animals. Ingestion or parenteral inoculation of the agent are the primary laboratory hazards. The oral ID_{25-50} of *S. flexneri* for humans is on the order of 200 organisms.[122] 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 not available for use in humans.

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.[90] Humans are the only known natural reservoir of the agent.

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 lesions and in blood. Accidental parenteral inoculation, and contact of mucous membranes or broken skin with infectious clinical materials, [and, perhaps, 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 in personnel regularly working with infectious materials. Vaccines are not available for use in humans.

Agent: **Vibrionic enteritis (*Campylobacter fetus,* subspecies *jejuni, Vibrio cholerae, Vibrio parahaemolyticus*)**

Vibrionic enteritis, due to *Campylobacter f. jejuni, Vibrio cholerae,* or *Vibrio parahaemolyticus,* is a documented but rare cause of laboratory-associated illnesses.[92] Naturally and experimentally infected animals are a potential source of infection.[94]

Laboratory Hazards: All pathogenic vibrios may occur in feces. *Campylobacter fetus* may also be present in blood, exudates from abscesses, tissues, and sputa. 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 of the order of 10^8 organisms.[94] 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 and facilities are recommended for activities with naturally or experimentally infected animals. Although vaccines have been shown to provide partial protection of short duration (3-6 months) to nonimmune individuals in highly endemic areas,[7] the routine use of cholera vaccine in laboratory staff is not recommended.

Agent: ***Yersinia pestis***

Plague is a proven but rare laboratory hazard. Four cases have been reported in the United States.[11,90]

Laboratory Hazards: The agent may be present in bubo fluid, blood, sputum, CSF, feces, and urine from humans, depending on the clinical form and stage of the disease. Direct contact with cultures and infectious materials from humans or rodents, infectious aerosols or droplets generated during the manipulation of cultures and infected tissues and in the necropsy of rodents, accidental autoinoculation, ingestion, and bites by infected fleas collected from rodents are the primary hazards to laboratory personnel.

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 of 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. Although field trials have not been conducted to determine the efficacy of licensed inactivated vaccines, experience with this product has been favorable.[14] Immunization is recommended for personnel working regularly with cultures of *Y. pestis* or infected rodents.[26]

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.

• *Rickettsial Agents*

Agent: ***Coxiella burnetii***

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.[90] 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 and often asymptomatic sheep and to their birth products is a documented hazard to personnel.[20,109] The agent is remarkably resistant to drying and is stable under a variety of environmental conditions.[121]

Laboratory Hazards: 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 10^9 organisms per gram of tissue, and milk may contain 10^5 organisms per gram. Parenteral inoculation and exposure to infectious aerosols and droplets are the most likely sources of infection to laboratory and animal care personnel. The estimated human ID_{25-50} (inhalation) for Q fever is 10 organisms.[122]

Recommended Precautions: Biosafety Level 2 practices, containment equipment, 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 embryonated eggs or tissue cultures, the necropsy of infected animals and the manipulation of infected tissues. Since infected guinea pigs and other rodents may shed the organisms in urine or feces,[90] experimentally infected rodents should be maintained under Animal Biosafety Level 3. Recommended precautions for facilities using sheep as experimental animals are described by Spinelli[109] and by Bernard.[6] An investigational new Phase I Q fever vaccine (IND) is available from the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), Fort Detrick, Maryland. The use of this vaccine should be limited to those at high risk of exposure who have no demonstrated sensitivity to Q fever antigen.

Agent: ***Rickettsia akari, Rochalimaea quintana,*** **and** ***Rochalimaea vinsonii***

Based on the experience of laboratories actively working with *Rickettsia akari*, it is likely that the 5 cases of rickettsial pox recorded by Pike[90] were associated with exposure to bites of infected mites rather than aerosol or contact exposure to infected tissues. There are no recorded cases of laboratory-associated infections with trench fever (*Rickettsia quintana*) or vole rickettsia (*R. vinsonii*).

Laboratory Hazard: The agent of rickettsial pox may be present in blood and other tissues of infected house mice or humans, and in the mite vector *Liponyssoides sanguineus.* Exposure to naturally or experimentally infected mites and accidental parenteral inoculation are the most likely sources of human infection with rickettsial pox. The agent of trench fever may be present in the blood and tissues of infected humans and in the body fluids and feces of infected human body lice (*Pediculus h. humanus*).

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for propagation and animal studies with *R. akari, R. vinsonii* and *R. quintana.* Appropriate precautions should be taken to avoid exposure of personnel to infected mites that are maintained in the laboratory or that may be present on naturally infected house mice.

Agent: ***Rickettsia prowazekii, Rickettsia typhi (R. mooseri), Rickettsia tsutsugamushi, Rickettsia canada,*** **and Spotted Fever Group agents of human disease other than** ***Rickettsia rickettsii*** **and** ***Rickettsia akari.***

Pike reported 57 cases of laboratory-associated typhus (type not specified), 56 cases of epidemic typhus with 3 deaths, and 68 cases of murine typhus.[90] More recently 3 cases of murine typhus were reported from a research facility.[18] Two of these 3 cases were associated with work with infectious materials on the open bench; the third case resulted from an accidental parenteral inoculation. These 3 cases represented an attack rate of 20% in personnel working with infectious materials.

Laboratory Hazard: Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of laboratory-associated infections. Naturally or experimentally infected lice, fleas and flying squirrels (*Glaucomys* spp)[9] may also be a direct source of infection to laboratory personnel. The organisms are relatively unstable under ambient environmental conditions.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for nonpropagative laboratory procedures, including serological and fluorescent antibody procedures and for the staining of impression smears. 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 embryonated eggs or tissue cultures. Animal Biosafety Level 2 practices and facilities are recommended for activities with infected mammals other than flying squirrels or arthropods. Vaccines are not currently available for use in humans. Because the mode of transmission of *Rickettsia prowazekii* from flying squirrels to humans is not defined, Animal Biosafety Level 3 practices and facilities are recommended for animal studies with flying squirrels naturally or experimentally infected with *R. prowazekii.*

Agent: ***Rickettsia rickettsii***

Rocky Mountain spotted fever is a documented hazard to laboratory personnel. Pike[90] reported 63 laboratory-associated cases, 11 of which were fatal. Oster[86] 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.

Laboratory Hazards: Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of laboratory-associated infection.[57] Successful aerosol transmission has been experimentally documented in nonhuman primates[101] Naturally and experimentally infected mammals, their ectoparasites, and their infected tissues are sources of human infection. The organism is relatively unstable under ambient environmental conditions.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all nonpropagative laboratory procedures, including serological and fluorescent antibody tests, and 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 embryonated eggs or tissue cultures. Animal Biosafety Level 2 practices and facilities are recommended for holding of experimentally infected rodents, however, necropsy and any subsequent manipulation of tissues from infected animals should be conducted at Biosafety Level 3.

Because of the proven value of antibiotic therapy in the early stages of infection, it is essential that laboratories working with *R. rickettsii* have an effective system for reporting febrile illnesses in laboratory personnel, medical evaluation of potential cases and, when indicated, institution of appropriate antibiotic therapy. Vaccines are not currently available for use in humans (see Appendix C).

• *Viral Agents*

Agent: **Hepatitis A Virus**

Laboratory-associated infections with hepatitis A virus 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 which are naturally or experimentally infected.[92]

Laboratory Hazards: The agent may be present in feces of infected humans and chimpanzees. 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 have not been fully defined but appear to result from seria passage in tissue culture.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with known or potentially infected feces from humans or chimpanzees. Animal Biosafety Level 2 practices and facilities are recommended for activities using naturally or experimentally infected chimpanzees. Animal care personnel should wear gloves and take other appropriate precautions to avoid possible fecal-oral exposure. Vaccines are not available for use in humans, but are in the developmental stages.

Agent: **Hepatitis B, Hepatitis nonA-nonB**

Pike concluded that hepatitis B is currently the most frequently occurring laboratory-associated infection.[90] The incidence in some categories of laboratory workers is seven times greater than that of the general population.[104] Epidemiological evidence indicates that hepatitis nonA-nonB is a blood-borne disease similar to hepatitis B.

Laboratory Hazard: The agent of hepatitis B 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 are not defined.

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 precautions, such as those described for Biosafety Level 3, may be indicated for activities with high 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. A licensed inactivated vaccine is available and is recommended for laboratory

personnel who are at substantially greater risk of acquiring infection than the general population.[27]

*Agent: **Herpesvirus simiae*** **(B-virus)**

Although B-virus presents a potential hazard to laboratory personnel working with the agent, laboratory-associated human infections with B-virus have, with rare exceptions, been limited to personnel having direct contact with living Old World monkeys.[29,56,89] Exposure to *in vitro* monkey tissues (i.e., primary rhesus monkey kidney) has been associated with a single documented case.[29].

B-virus is an indigenous chronic and/or recurrent infection of macaques and possibly other Old World monkeys and is a frequent enzootic infection of captive *Macaca mulatta*.

Laboratory personnel handling Old World monkeys run the risk of acquiring B-virus from a bite or contamination of broken skin or mucous membranes by an infected monkey. Fifteen fatal cases of human infections with B-virus have been reported.[29]

Laboratory Hazards: The agent may be present in oral secretions, thoracic and abdominal viscera, and central nervous system tissues of naturally infected macaques. Bites from monkeys with oral herpes lesions are the greatest hazard to laboratory and animal care personnel. Exposures of broken skin or mucous membranes to oral secretions or to infectious culture fluids are also potential hazards. The importance of aerosol exposure is not known. Attenuated or avirulent strains have not been defined.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of tissues, body fluids, and primary tissue culture materials from macaques. Additional containment and personnel precautions, such as those recommended for Biosafety Level 3, are recommended for activities involving the use or manipulation of any material known to contain *Herpesvirus simiae*.

Biosafety Level 4 practices, containment equipment, and facilities are recommended for activities involving the propagation of *H. simiae*, manipulations of production quantities or concentrations of *H. simiae* and housing vertebrate animals with proven natural or induced infection with the agent.

The wearing of gloves, masks, and laboratory coats is recommended for all personnel working with nonhuman primates—especially macaques and other Old World species—and for all persons entering animal rooms where nonhuman primates are housed. Vaccines are not available for use in humans.

*Agent: **Herpesviruses***

The herpesviruses are ubiquitous human pathogens and are commonly present in a variety of clinical materials submitted for virus isolation. While these viruses are not demonstrated causes of laboratory-associated

infections, they are primary, as well as opportunistic pathogens—especially in immuno-compromised hosts. Nonpolio enteroviruses, adenoviruses, and cytomegalovirus pose similar low potential infection risks to laboratory personnel. 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, nonpolio enteroviruses, and other indigenous pathogens 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.

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 which are associated or identified as a primary pathogen of human disease. Although there is no definitive 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, 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.

Agent: **Influenza**

Laboratory-associated infections with influenza are not normally documented in the literature, but are known to occur by informal accounts and published reports, particularly when new strains showing antigenic drift or shift are introduced into a laboratory for diagnostic/research purposes.[36]

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 man 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.

Inhalation of virus from aerosols generated when aspirating, dispensing, or mixing virus-infected samples, or by infected animals are the primary laboratory hazards. Genetic manipulation of virus has unknown potential for altering host range, pathogenicity or for introducing into man transmissible viruses with novel antigenic composition.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, 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 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. Current experience suggests, however, there is no evidence for laboratory-acquired infection with reference strains A/PR/8/34 and A/WS/33 or its commonly used neurotropic variants.

Agent: **Lymphocytic Choriomeningitis (LCM) Virus**

Laboratory-associated infections with LCM virus are well documented in facilities where infections occur in laboratory rodents—especially mice and hamsters.[8,90] Tissue cultures which have inadvertently become infected represent a potential source of infection and dissemination of the agent. Natural infections are occasionally found in nonhuman primates, swine, and dogs.

Laboratory Hazards: The agent may be present in blood, CSF, urine, secretions of the nasopharynx, feces and tissues of infected humans and other animal hosts. 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.[8]

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids or tissues and for tissue culture passage of mouse brain-passaged strains. All manipulations of known or potentially infectious passage and clinical materials should be conducted in a biological safety cabinet. Additional primary containment and personnel precautions such as those described for Biosafety Level 3 may be indicated for activities with high potential for aerosol production and for activities involving production quantities or concentrations of infectious materials. Animal Biosafety Level 2 practices and facilities are recommended for studies in adult mice with mouse brain-passaged strains . Animal Biosafety Level 3 practices and facilities are recommended for work with infected hamsters. Vaccines are not available for use in humans.

Agent: **Poliovirus**

Laboratory-associated infections with polioviruses are uncommon and are generally limited to unvaccinated laboratory persons working directly with the agent. Twelve cases have been reported in the world literature.[90]

Laboratory animal-associated infections have not been reported;[73] however, naturally or experimentally infected nonhuman primates could provide a source of infection to exposed unvaccinated persons.

Laboratory Hazards: The agent may be found in the feces and in throat secretions. Ingestion or parenteral inoculation of infectious tissues or fluids by unimmunized personnel are the primary hazards to laboratory personnel. The importance of aerosol exposure is not known. Laboratory exposures pose negligible risk to appropriately immunized persons.

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities recommended for all activities utilizing known or potentially infectious culture fluids and specimen materials. All laboratory personnel working directly with the agent must have documented polio vaccination or demonstrated serologic evidence of immunity to all three poliovirus types.[25]

Agent: **Poxviruses**

Sporadic cases of laboratory-associated poxvirus infections have been reported. Pike lists 24 cases of yaba and tanapox virus infection and 18 vaccinia and smallpox infections[90] Epidemiological evidence suggests that transmission of monkeypox virus from nonhuman primates or rodents 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.

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, Atlanta, Georgia. Biosafety Level 2 practices, containment equipment, 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 preceeding three years.[23] Activities with vaccinia, cow pox, or monkey pox viruses in quantities or concentrations greater than those present in diagnostic cultures may also be conducted by immunized personnel at Biosafety Level 2, provided that all manipulations of viable materials are conducted in Class I or II biological safety cabinets or other primary containment equipment.

Agent: **Rabies Virus**

Laboratory-associated infections are extremely rare. Two have been documented. Both resulted from presumed exposure to high-titered infectious aerosols generated in a vaccine production facility[129] and a research

facility.[17]. Naturally or experimentally infected animals, their tissues, and their excretions are a potential source of exposure to laboratory and animal care personnel.

Laboratory Hazards: The agent may be present in all tissues of infected animals. Highest titers are demonstrated in central nervous system 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 droplets of tissue or fluids are the most likely sources of exposure for 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.[17,129]

Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials. Preexposure immunization is recommended for all individuals working with rabies virus, infected animals, or engaged in diagnostic, production, or research activities with rabies virus. Preexposure 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 feasible to open the skull or remove the brain 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 striking the brain with the blade of the 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.

Agents: Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob and kuru agents)

Laboratory-associated infections with the transmissible spongiform encephalopathies have not been documented. The consequences of infection are grave, however, and there is evidence that Creutzfeldt-Jakob disease (CJD) has been transmitted to patients by corneal transplant and by contaminated electroencephalographic electrodes. 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.

Laboratory Hazards: High titers of a transmissible agent have been demonstrated in brain and spinal cord of persons with kuru. In persons with

Creutzfeldt-Jakob disease, a transmissible agent has been demonstrated in the brain, spleen, liver, lymph nodes, lungs, spinal cord, kidneys, cornea and lens. Accidental parenteral inoculation, especially neural tissues, and including formalin-fixed specimens, is extremely hazardous. Although non-neural tissues are less often infective, 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, and chemicals (formalin, betapropiolactone, alcohols).

Recommended Precautions: Biosafety Level 2 practices, containmen equipment, 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.[46] 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, fluids, and during the necropsy of experimental animals. It is further recommended that gloves should be worn for activities which provide the opportunity for skin contact with infectious tissues and fluids. Vaccines are not available for use in humans.

Agent: **Vesicular Stomatitis Virus (VSV)**

Forty-six laboratory-associated infections with indigenous strains of VSV have been reported.[112] Laboratory activities with indigenous strains of VSV 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.[52,88] Seroconversion and clinical illness rates in personnel working with these materials are high.[88] Similar risks may be associated with exotic strains such as Piry.[112]

In contrast, anecdotal information indicates that activities with less virulent laboratory-adapted strains (e.g., VSV-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. 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, containment equipment, 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.

Arboviruses

• *Arboviruses Assigned to Biosafety Level 2*

The American Committee on Arthropod-Borne Viruses (ACAV) registered 424 arboviruses as of December 31, 1979. The ACAV's Subcommittee on Arbovirus Laboratory Safety (SALS) has categorized each of these 424 agents into 1 of 4 recommended levels of practice and containment which parallel the recommended practices, safety equipment, and facilities described in this publication as Biosafety Levels 1-4.[112] It is the intent of SALS to periodically update the 1980 publication by providing a supplemental listing and recommended levels of practice and containment for arboviruses registered since 1979. SALS categorizations were based on risk assessments from information provided by a worldwide survey of 585 laboratories working with arboviruses. SALS recommended that work with the majority of these agents should be conducted at the equivalent of Biosafety Level 2. These viruses are listed alphabetically on pages 66 through 68 and include the following agents which are the reported cause of laboratory-associated infections.[53,90,112] The list of arboviruses in Biosafety Level 2 includes yellow fever virus (17D strain) and Venezuelan Equine Encephalomyelitis (VEE) virus (TC83 strain), provided that personnel working with these vaccine strains are immunized.

Virus	**Cases (SALS)**
Vesicular stomatitis	46
Colorado tick fever	16
Dengue	11
Pichinde	17
Western equine encephalomyelitis	7 (2 deaths)
Rio Bravo	7
Kunjin	6
Catu	6
Caraparu	5
Ross River	5
Bunyamwera	4
Eastern equine encephalomyelitis	4
Zika	4
Apeu	2
Marituba	2
Tacaribe	2
Muructucu	1
O'nyong nyong	1
Modoc	1
Oriboca	1
Ossa	1
Keystone	1
Bebaru	1
Bluetongue	1

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. Recommendations that work with these 334 arboviruses should be conducted at Biosafety Level 2 was based on the existence of adequate historical laboratory experience to assess risks for the virus which indicated that (a) no overt laboratory-associated infections are reported or (b) infections resulted from exposures other than to infectious aerosols or (c) if aerosol exposures are documented they represent an uncommon route of exposure.

Laboratory Hazard: Agents listed in this group may be present in blood, CSF, central nervous system and other tissues, and infected arthropods, depending on the agent and the stage of infection. While the primary laboratory hazards are accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites of infected laboratory rodents or arthropods, 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 potentially infectious clinical materials and arthropods and for manipulations of infected tissue cultures, embryonated eggs, and rodents. Infection of newly hatched chickens with eastern and western equine encephalomyelitis viruses is especially hazardous and should be undertaken under Biosafety Level 3 conditions by immunized personnel. Investigational vaccines (IND) against eastern equine encephalomyelitis and western equine encephalomyelitis viruses are available through the Centers for Disease Control and the U.S. Army Medical Research Institute for Infectious Diseases, respectively. The use of these vaccines is recommended for personnel who work directly and regularly with these two agents in the laboratory. Western equine encephalomyelitis immune globulin (human) is also available from the Centers for Disease Control. The efficacy of this product has not been established.

Arboviruses Assigned to Biosafety Level 2

Abu Hammad
Acado
Acara
Aguacate
Alfuy
Almpiwar
Amapari
Anhanga
Anhembi
Anopheles A
Anopheles B
Apeu
Apoi
Aride
Arkonam
Aruac
Arumowot
Aura
Avalon
Bagaza
Bahig
Bakau
Baku
Bandia
Bangoran
Bangui
Banzi
Barur
Batai
Batu
Bauline
Bebaru
Belmont
Bertioga
Bimiti
Birao
Bluetongue (indigenous)
Boraceia
Botambi
Boteke
Bouboui
Bujaru
Bunyamwera
Burg el Arab
Bushbush
Bussuquara
Buttonwillow
Bwamba

Arboviruses Assigned to Biosafety Level 2 (continued)

Cacao
Cache Valley
Caimito
California Encephalitis
Calovo
Candiru
Cape Wrath
Capim
Caraparu
Carey Island
Catu
Chaco
Chagres
Chandipura
Changuinola
Charleville
Chenuda
Chilibre
Chobar Gorge
Clo Mor
Colorado Tick Fever
Corriparta
Cotia
Cowbone Ridge
D'Aguilar
Dakar Bat
Dengue-1
Dengue-2
Dengue-3
Dengue-4
Dera Ghazi Khan
Eastern Equine Encephalomyelitis
Edge Hill
Entebbe Bat
Epizootic Hemorrhagic Disease
Eubenangee
Eyach
Flanders
Fort Morgan
Frijoles
Gamboa
Gomoka
Gossas
Grand Arbaud
Great Island
Guajara
Guama
Guaroa
Gumbo Limbo
Hart Park
Hazara
Huacho
Hughes
Icoaraci
Ieri
Ilesha
Ilheus
Ingwavuma
Inkoo
Ippy
Irituia
Isfahan
Itaporanga
Itaqui
Jamestown Canyon
Japanaut
Jerry Slough
Johnston Atoll
Joinjakaka
Juan Diaz
Jugra
Jurona
Jutiapa
Kadam
Kaeng Khoi
Kaikalur
Kaisodi
Kamese
Kammavanpettai
Kannamangalam
Kao Shuan
Karimabad
Karshi
Kasba
Kemerovo
Kern Canyon
Ketapang
Keterah
Keuraliba
Keystone
Klamath
Kokobera
Kolongo
Koongol
Kowanyama
Kunjin
Kununurra
Kwatta
La Crosse
Lagos Bat
La Joya
Landjia
Langat
Lanjan
Latino
Lebombo
Le Dantec
Lipovnik
Lokern
Lone Star
Lukuni
M'Poko
Madrid
Maguari
Mahogany Hammock
Main Drain
Malakal
Manawa
Manzanilla
Mapputta
Maprik
Marco
Marituba
Matariya
Matruh
Matucare
Melao
Mermet
Minatitlan
Minnal
Mirim
Mitchell River
Modoc
Moju
Mono Lake
Mont. Myotis Leuk.
Moriche
Mossuril
Mount Elgon Bat
Murutucu
Navarro
Nepuyo
Ngaingan
Nique
Nkolbisson
Nola
Ntaya
Nugget
Nyamanini
Nyando
O'nyong-nyong
Okhotskiy
Okola
Olifantsvlei
Oriboca
Ossa
Pacora
Pacui
Pahayokee
Palyam
Parana
Pata
Pathum Thani

Arboviruses Assigned to Biosafety Level 2 (continued)

Patois
Phnom-Penh Bat
Pichinde
Pixuna
Pongola
Pretoria
Puchong
Punta Salinas
Punta Toro
Qalyub
Quaranfil
Restan
Rio Bravo
Rio Grande
Ross River
Royal Farm
Sabo
Saboya
Saint Floris
Sakhalin
Salehabad
San Angelo
Sandfly F. (Naples)
Sandfly F. (Sicilian)
Sandjimba
Sathuperi
Sawgrass
Sebokele
Seletar
Sembalam
Shamonda
Shark River
Shuni
Silverwater
Simbu
Simian Hemorrhagic Fever
Sindbis
Sixgun City
Snowshoe Hare
Sokuluk
Soldado
Sororoca
Stratford
Sunday Canyon
Tacaiuma
Tacaribe
Taggert
Tahyna
Tamiami
Tanga
Tanjong Rabok
Tataguine
Tembe
Tembusu
Tensaw
Tete
Tettnang
Thimiri
Thottapalayam
Timbo
Toure
Tribec
Triniti
Trivittatus
Trubanaman
Tsuruse
Turlock
Tyuleniy
Uganda S
Umatilla
Umbre
Una
Upolu
Urucuri
Usutu
Uukuniemi
Vellore
Venezuelan Equine Encephalomyelitis (TC-83)
Venkatapuram
Vesicular Stomatitis*
Wad Medani
Wallal
Wanowrie
Warrego
Western Equine Encephalomyelitis
Whataroa
Witwatersrand
Wongal
Wongorr
Wyeomyia
Yaquina Head
Yata
Yellow Fever (17D)
Yogue
Zaliv Terpeniya
Zegla
Zika
Zingilamo
Zirqa

*See Agent Summary Statement on page 68.

• *Arboviruses and Arenaviruses Assigned to Biosafety Level 3*

SALS has recommended that work with the arboviruses included in the alphabetical listing on page 70 should be conducted at the equivalent of Biosafety Level 3 practices, safety equipment and facilities. These recommendations are based on one of the following criteria: overt laboratory-associated infections with these agents have occurred by aerosol route if protective vaccines are not used or are unavailable; or laboratory experience with the agent is inadequate to assess risk and the natural disease in humans is potentially severe, life threatening, or causes residual damage. Hantaan virus, which was not included in the SALS publication, has been placed at Biosafety Level 3 based on documented laboratory-associated infections. Rift Valley Fever virus, which was classified by SALS at Containment Level 3* (i.e., HEPA filtration required for all air exhausted from the laboratory) was placed in Biosafety Level 3 provided that all personnel entering the laboratory or animal care area where work with this virus is being conducted are vaccinated. Laboratory or laboratory animal-associated infections have been reported with the following agents:[53,90,112,124,]

Virus	Cases (SALS)
Venezuelan equine encephalitis	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 (Korean hemorrhagic fever)	6
Mayaro	5
Spondweni	4
St. Louis encephalitis	4
Murray Valley encephalitis	3
Semliki Forest	3 (1 death)
Powassan	2
Dugbe	2
Issyk-kul	1
Koutango	1

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.[126] Because this infection may have been influenced by a compromised host, an unusual route of exposure or high dosage, or a mutated strain of the virus, this case and its outcome may not be typical. Since exposure to an infectious aerosol was not indicated as the probable mode of transmission in this case, it is suggested that most activities with Semliki Forest disease virus can be safely conducted at Biosafety Level 2.

Some viruses (e.g., Ibaraki, Israel Turkey Meningoencephalitis) are listed by SALS in Level 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, CSF, urine and exudates, depending on the specific agent and stage of disease. The primary laboratory hazards are exposure to aerosols of infectious solutions and animal bedding, accidental parenteral inoculation, and broken skin contact. Some of these agents (e.g., VEE) may be relatively stable in dried blood or exudates. Attenuated strains are identified in a number of the agents listed (e.g., yellow fever-17D strain and VEE-TC83 strain).

Recommended Precautions: Biosafety Level 3 practices, containment equipment, and facilities are recommended for activities using potentially infectious clinical materials and infected tissue cultures, animals, or arthropods.

A licensed attenuated live virus is available for immunization against yellow fever and is recommended for all personnel who work with this agent or with infected animals, and those who enter rooms where the agents or infected animals are present. An investigational vaccine (IND) is available for immunization against VEE and is recommended for all personnel working with VEE (and the related Everglades, Mucambo, Tonate, and Cabassou viruses), infected animals, or entering rooms where these agents or infected animals are present. Work with Hantaan (Korean hemorrhagic fever) virus in rats, voles, and other laboratory rodents should be conducted with special caution (Biosafety Level 4). An inactivated, investigational new Rift Valley fever vaccine (IND) is available from the U.S. Army Medical Research Institute for Infectious Diseases (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.

Arboviruses Assigned to Biosafety Level 3:

Aino
Akabane
Araguari
Batama
Batken
Bhanja
Bimbo
Bluetongue (exotic)[a]
Bobaya
Bobia
Buenaventura
Cabassou[c]
Chikungunya[c]
Chim
Cocal
Dhori
Dugbe
Everglades[c]
Garba
Germiston[c]
Getah
Gordil
Guaratuba
Ibaraki
Ihangapi
Inini
Israel Turkey Meningo.
Issyk-Kul
Itaituba
Japanese encephalitis
Kairi
Khasan
Korean Hemorrhagic Fever (Hantaan)
Koutango
Kyzylagach
Louping Ill[a]
Lymphocytic choriomeningitis
Mayaro
Middelburg
Mosqueiro
Mucambo[c]
Murray Valley encephalitis
Nariva
Ndumu
Negishi
New Minto
Nodamura
Northway
Oropouche[c]
Orungo
Ouango
Oubangui
Paramushir
Piry
Ponteves
Powassan
Razdan
Rift Valley fever[a,b,c]
Rochambeau
Rocio[c]
Sagiyama
Sakpa
Salanga
Santa Rosa
Saumarez Reef
Semliki Forest
Sepik
Serra do Navio
Slovakia
Spondweni
St. Louis encephalitis
Tamdy
Telok Forest
Thogoto
Tlacotalpan
Tonate[c]
VSV-Alagoas
Venezuelan equine encephalomyelitis[c]
Wesselsbron[a,c]
West Nile
Yellow Fever[c]
Zinga[a,b,c]

[a]The importation, possession, or use of this agent is restricted by USDA regulation or administrative policy. See Appendix E.

[b]Zinga virus is now recognized as being identical to Rift Valley Fever virus.

[c]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. All persons working with agents for which a vaccine is available should be immunized.

• *Arboviruses, Arenaviruses, or Filoviruses Assigned to Biosafety Level 4*

SALS has recommended that work with the arboviruses, arenaviruses, or filoviruses[63] included in the listing that follows should 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. Additionally, SALS recommended that certain agents with a close or identical antigenic relationship to the Biosafety Level 4 agents (e.g., Absettarov and Kumlinge viruses) also be handled at this level until sufficient laboratory experience is obtained to retain these agents at this level or to work with them at a lower level. Laboratory or laboratory animal-associated infections have been reported with the following agents:[37,53,58,67,90,112,124,]

Virus	Cases (SALS)
Kyasanur Forest Disease	133
Hypr	37 (2 deaths)
Junin	21 (1 death)
Marburg	25 (5 deaths)
Russian Spring-Summer encephalitis	8
Congo-Crimean hemorrhagic fever	8(1 death)
Omsk hemorrhagic fever	5
Lassa	2 (1 death)
Machupo	1 (1 death)
Ebola	1

Rodents are natural reservoirs of Lassa fever virus (*Mastomys natalensis*), Junin and Machupo viruses (*Calomys* spp.) and perhaps other viruses assigned to Biosafety Level 4. Nonhuman primates were associated with the initial outbreaks of Kyasanur Forest disease (*Presbytis* spp.) and Marburg disease (*Cercopithecus* spp.), and 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.[67,124]

Recommended Precautions: Biosafety Level 4 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials of human, animal, or arthropod origin. Clinical specimens from persons suspected of being infected with one of the agents listed in this summary should be submitted to a laboratory with a Biosafety Level 4 maximum containment facility.[22]

Arboviruses, Arenaviruses and Filoviruses Assigned to Biosafety Level 4:

Congo-Crimean hemorrhagic fever
Tick-borne encephalitis virus complex
(Absettarov, Hanzalova, Hypr,
Kumlinge, Kyasanur Forest disease,
Omsk hemorrhagic fever, and
Russian Spring-Summer encephalitis)
Marburg
Ebola
Junin
Lassa
Machupo

Appendix A
Biological Safety Cabinets

Biological safety cabinets are among the most effective, as well as the most commonly used, primary containment devices in laboratories working with infectious agents. Each of the three types—Class I, II, and III—has performance characteristics which are described in this appendix. In addition to the design, construction, and performance standards for vertical laminar flow biological safety cabinets (Class II), the National Sanitation Foundation has also developed a list of such products which meet the reference standard. Utilization of this standard[80] and list should be the first step in selection and procurement of a biological safety cabinet.

Class I and II biological safety cabinets, when used in conjunction with good microbiological techniques, provide an effective partial containment system for safe manipulation of moderate and high-risk microorganisms (i.e., Biosafety Level 2 and 3 agents). Both Class I and II biological safety cabinets have comparable inward face velocities (75 linear feet per minute) and provide comparable levels of containment in protecting the laboratory worker and the immediate laboratory environment from infectious aerosols generated within the cabinet.

It is imperative that Class I and II biological safety cabinets are 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. The slide-sound training film developed by NIH (Effective Use of the Laminar Flow Biological Safety Cabinet) provides a thorough training and orientation guide. Of particular note are those activities which may disrupt the inward directional airflow through the work opening of Class I and II cabinets. Repeated insertion and withdrawal of the workers' arms in and from 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 are demonstrated causes of the escape of aerosolized particles from within the cabinet. Strict adherence to recommended practices for the use of biological safety cabinets is as important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself.

Horizontal laminar flow "clean benches" are present in a number of clinical, pharmacy, and laboratory facilities. These "clean benches" provide a high quality environment within the work chamber for manipulation of nonhazardous materials. *Caution*: Since the operator sits in the immediate downstream exhaust from the "clean bench," this equipment must never be used for the handling of toxic, infectious, or sensitizing materials.

The *Class I* biological safety cabinet is an open-fronted, negative-pressure, ventilated cabinet with a minimum inward face velocity at the work opening of at least 75 feet per minute. The exhaust air from the cabinet is filtered by a high efficiency particulate air (HEPA) filter. This cabinet may be used in three operational modes: with a full-width open front, with an installed front closure panel not equipped with gloves, and with an installed front closure panel equipped with arm-length rubber gloves.

The *Class II* vertical laminar-flow biological cabinet is an open-fronted, ventilated cabinet with an average inward face velocity at the work opening of at least 75 feet per minute. This cabinet provides a HEPA-filtered, recirculated mass airflow within the work space. The exhaust air from the cabinet is also filtered by HEPA filters. Design, construction, and performance standards for Class II cabinets have been developed by and are available from the National Sanitation Foundation, Ann Arbor, Michigan.[19]

The *Class III* cabinet is a totally enclosed ventilated cabinet of gas-tight construction. Operations within the Class III cabinet are conducted through attached rubber gloves. When in use, the Class III cabinet is maintained under negative air pressure of at least 0.5 inches water gauge. Supply air is drawn into the cabinet through HEPA filters. The cabinet exhaust air is filtered by two HEPA filters, installed in series, before discharge outside of the facility. The exhaust fan for the Class III cabinet is generally separate from the exhuast fans of the facility's ventilation system.

Personnel protection provided by Class I and Class II cabinets is dependent on the inward airflow. Since the face velocities are similar, they generally provide an equivalent level of personnel protection. The use of these cabinets alone, however, is not appropriate for containment of highest-risk infectious agents because aerosols may accidentally escape through the open front.

The use of a Class II cabinet in the microbiological laboratory offers the additional capability and advantage of protecting materials contained within it from extraneous airborne contaminants. This capability is provided by the HEPA-filtered, recirculated mass airflow within the work space.

The Class III cabinet provides the highest level of personnel and product protection. This protection is provided by the physical isolation of the space in which the infectious agent is maintained. When these cabinets are required, all procedures involving infectious agents are contained within them. Several Class III cabinets are therefore typically set up as an interconnected system. All equipment required by the laboratory activity, such as incubators, refrigerators, and centrifuges, must be an integral part of the cabinet system. Double-doored autoclaves and chemical dunk tanks are also attached to the cabinet system to allow supplies and equipment to be safely introduced and removed.

Personnel protection equivalent to that provided by Class III cabinets can also be obtained with a personnel suit area and Class I or Class II cabinets.

This is one in which the laboratory worker is protected from a potentially contaminated environment by a one-piece positive pressure suit ventilated by a life-support system. This area is entered through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surfaces of the suit as the worker leaves the area. The exhaust air from the suit area is filtered by two HEPA filter units installed in series.

FIGURE 1. Class I Cabinet

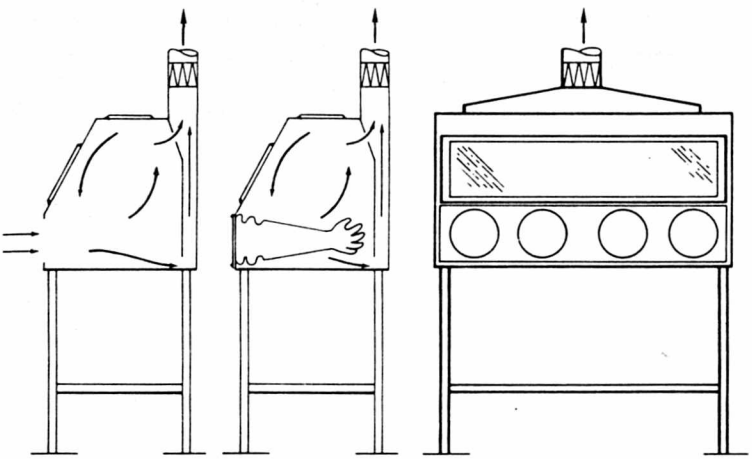
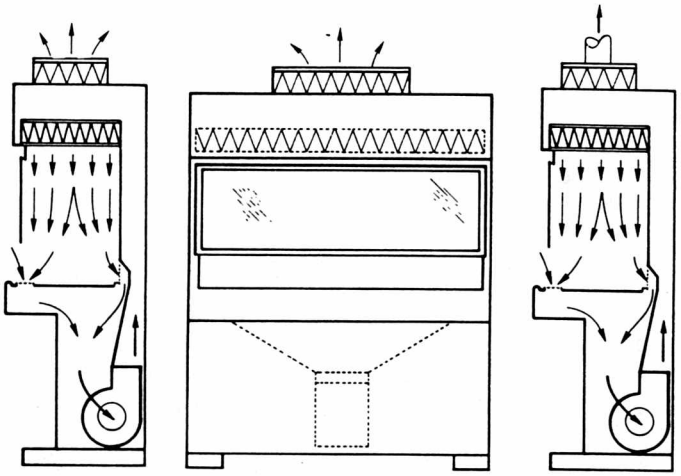
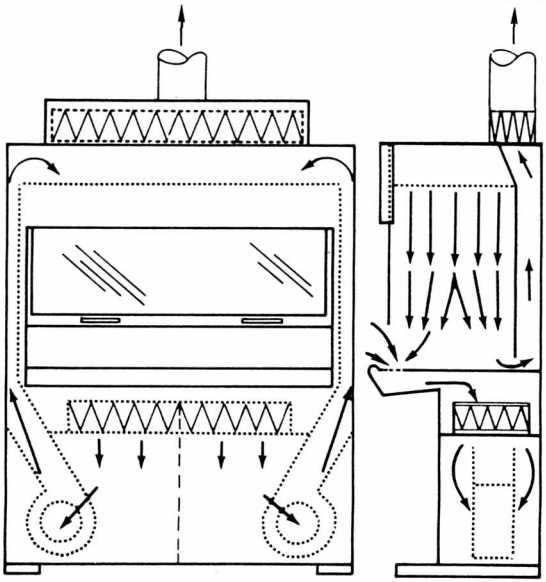


FIGURE 2. Class II Cabinets

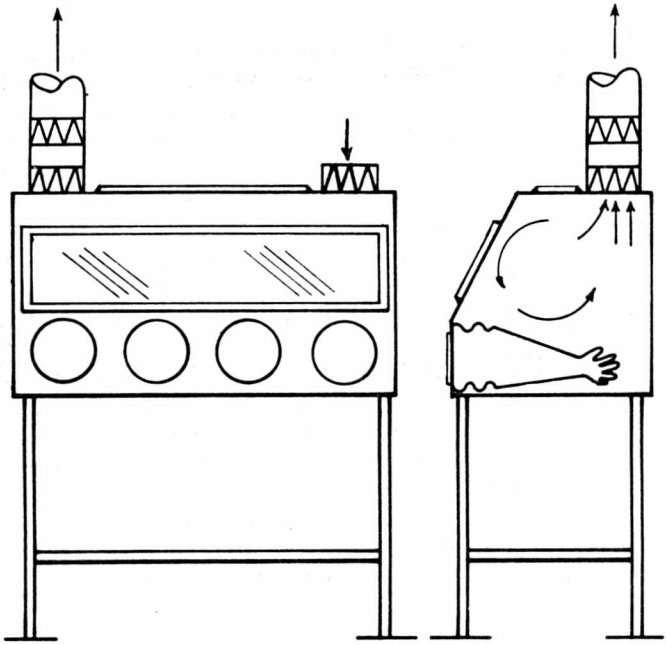


Type A



Type B

FIGURE 3. Class III Cabinet



Appendix B
Immunoprophylaxis

An additional level of protection for at-risk personnel may be achieved with appropriate prophylactic vaccinations. A written organizational policy which defines at-risk personnel, which specifies risks as well as benefits of specific vaccines, and which 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.

Vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risks (local or systemic reactions) should be required for all clearly identified at-risk personnel. Examples of such preparations include vaccines against yellow fever, rabies, and poliomyelitis. Recommendations for giving less efficacious vaccines,those associated with high rates of local or systemic reactions, or those that produce increasingly severe reactions with repeated use should be carefully considered. Products with these characteristics (e.g., cholera, tularemia, and typhoid vaccines) may be recommended but should not ordinarily be required for employment. A complete record of vaccines received on the basis of occupational requirements or recommendations should be maintained in the 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 V.

Appendix C

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 which might produce unusual and perhaps very serious responses are possible. Surveillance of personnel for laboratory-associated infections with rickettsial agents can dramatically reduce the risk of serious consequences of disease.

Recent experience indicates that infections treated adequately 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 into 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, prostration, when no other certain cause exists and, (5) a nonpunitive 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 who are knowledgeable about the hazards of rickettsial infections and who put to use the safeguards recommended in the agent summary statements.

Appendix D

Importation and Interstate Shipment of Human Pathogens and Related Materials

The importation or subsequent receipt of etiologic agents and vectors of human disease is subject to the Public Health Service Foreign Quarantine Regulations (42 CFR, Section 71.56). Permits authorizing the importation or receipt of regulated materials and specifying conditions under which the agent or vector is shipped, handled, and used are issued by the Centers for Disease Control.

The interstate shipment of indigenous etiologic agents, diagnostic specimens, and biological products is subject to applicable packaging, labeling, and shipping requirements of the Interstate Shipment of Etiologic Agents (42 CFR Part 72). Packaging and labeling requirements for interstate shipment of etiologic agents are summarized and illustrated in Figure 4.

Additional information on the importation and interstate shipment of etiologic agents of human disease and other related materials may be obtained by writing to:

Centers for Disease Control
Attention: Office of Biosafety
1600 Clifton Road, N.E.
Atlanta, Georgia 30333
Telephone: (404) 329-3883
FTS: 236-3883

FIGURE 4.

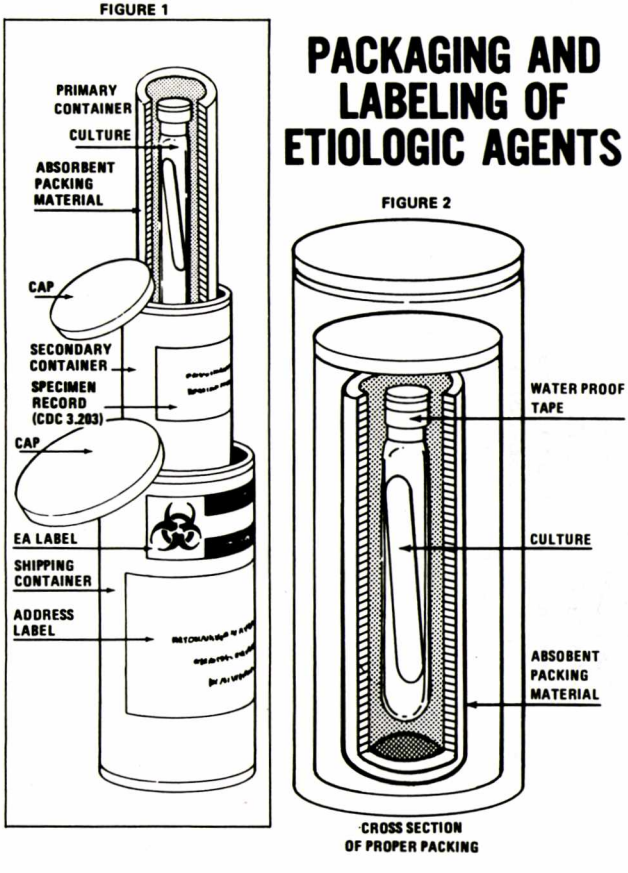


FIGURE 1
PRIMARY CONTAINER
CULTURE
ABSORBENT PACKING MATERIAL
CAP
SECONDARY CONTAINER
SPECIMEN RECORD (CDC 3.283)
CAP
EA LABEL
SHIPPING CONTAINER
ADDRESS LABEL
PACKAGING AND LABELING OF ETIOLOGIC AGENTS
FIGURE 2
WATER PROOF TAPE
CULTURE
ABSOBENT PACKING MATERIAL
CROSS SECTION OF PROPER PACKING

The Interstate Shipment of Etiologic Agents (42 CFR, Part 72) was revised July 21, 1980 to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic.

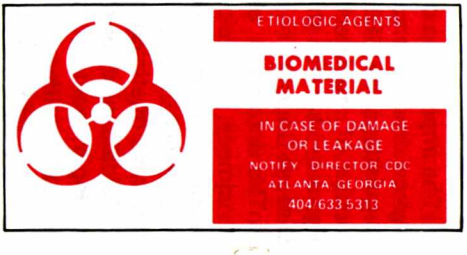
Figures 1 and 2 diagram the packaging and labeling of etiologic agents in volumes of less than 50 ml. in accordance with the provisions of subparagraph 72.3 (a) of the cited regulation. Figure 3 illustrates the color and size of the label, described in subparagraph 72.3 (d) (1 - 5) of the regulations, which shall be affixed to all shipments of etiologic agents.

For further information on any provision of this regulation contact:

Centers for Disease Control
Attn: Biohazards Control Officer
1600 Clifton Road
Atlanta, Georgia 30333

Telephone: 404—329-3883
FTS—236-3883

FIGURE 3



Appendix E
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 virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine ephemeral fever
Bovine infectious petechial fever agent
Camelpox virus
Foot and mouth disease virus
Fowl plague virus
Histoplasma (Zymonema) farciminosum
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Mycoplasma agalactiae
Mycoplasma mycoides
Nairobi sheep disease virus (Ganjam virus)
Newcastle disease virus (velogenic strains)
Pseudomonas mallei
Rickettsia ruminantium
Rift Valley fever virus
Rinderpest virus
Swine vesicular disease virus
Teschen disease virus
Theileria annulata
Theileria bovis
Theileria hirci
Theileria lawrencei
Trypanosoma evansi
Trypanosoma vivax
Vesicular exanthema virus
Wesselsbron disease virus

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.

Additional information may be obtained by writing to:

Chief Staff Veterinarian
Organisms and Vectors
Veterinary Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Hyattsville, Maryland 20782
Telephone: (301) 436-8017
FTS: 436-8017

Appendix F
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
Attention: Office of Biosafety
Atlanta, Georgia 30333
Telephone: (404) 329-3883
FTS 236-3883

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

National Animal Disease Center
U.S. Department of Agriculture
Ames, Iowa 50010
Telephone: (515) 862-8258
FTS 862-8258

References

1. Allen, B.W. 1981. Survival of tubercle bacilli in heat-fixed sputum smears. J Clin Pathol *34*:719-722.
2. American Thoracic Society Policy Statement. 1974. Quality of Laboratory Services for Mycobacterial Disease, Am Rev Respir Dis *110*:376-377.
3. Anon. 1980. Tuberculosis infection associated with tissue processing. Calif Morbidity 30.
4. Bacteriologist dies of meningitis. 1936. JAMA *106*:129.
5. Beeman, E.A. 1950. Q fever—An epidemiological note. 1950. Public Health Rep *65*,(2):88-92.
6. Bernard, K.W., Parham, G.L., Winkler, W.G., Helmick, C.G. 1982. Q fever control measures: Recommendations for research of facilities using sheep. Infect Control *3*,(6).
7. Blaser, M.J., Hickman, F.W., Farmer, J.J. III, Brenner, D.J., Balows, A. and Feldman, R.A. 1980. *Salmonella typhi*: The laboratory as a reservoir of infection. J Infect Dis *142*:934-938.
8. Bowen, G.S., Calisher, C.H., Winkler, W.G., Kraus, A.L., Fowler, E.H., Garman, R.H., Fraser, D.W., Hinman, A.R. 1975. Laboratory studies of a lymphocytic choriomeningitis virus outbreak in man and laboratory animals. Am J Epidemiol *102*:233-40.
9. Bozeman, F.M., Masiello, S.A., Williams, M.S., and Elisberg, B.L. 1975. Epidemic typhus rickettsiae isolated from flying squirrels. Nature *255*:545-547.
10. Burke, D.S. 1977. Immunization against tularemia: Analysis of the effectiveness of live *Francisella tularensis* vaccine in prevention of laboratory-acquired tularemia. J Infect Dis *135*:55-60.
11. Burmeister, R.W., Tigertt, W.D., and Overholt, E.L. 1962. Laboratory-acquired pneumonic plague. Ann Intern Med *56*:789-800.
12. Bush, J.D. 1943. Coccidioidomycosis. J Med Assoc Alabama *13*:159-166.
13. Carougeau, M. 1909. Premier cas Africain de sporotrichose de deBeurmann: Transmission de la sporotrichose du mulet a l'homme. Bull Mem Soc Med Hop (Paris) *28*:507-510.
14. Cavenaugh, D.C., Elisberg, B.L., Llewellyn, C.H., Marshall, J.D., Jr., Rust, J.H., Williams, J.E., and Meyer, K.F. 1974. Plague Immunization IV. Indirect Evidence for the efficacy of plague vaccine. J Infect Dis *129*: (Supplement) S37-S40.
15. Center for Disease Control, Office of Biosafety. 1974. Classification of Etiologic Agents on the Basis of Hazard, 4th Edition. U.S. Department of Health, Education and Welfare, Public Health Service.
16. Center for Disease Control. 1976. Unpublished Data. Center for Infectious Diseases. U.S. Department of Health, Education and Welfare, Public Health Service.
17. Center for Disease Control. 1977. Rabies in a laboratory worker New York. Morbidity and Mortality Weekly Report *26*(22):183-184.
18. Center for Disease Control. 1978. Laboratory-acquired endemic typhus. Morbidity and Mortality Weekly Report *27*(26):215-216.
19. Center for Disease Control. 1978. Meningococcal Polysaccharide vaccines. Recommendations of the Immunization Practices Advisory Committee (ACIP). Morbidity and Mortality Weekly Report *27*(35):327-328.
20. Centers for Disease Control. 1979. Q fever at a university research center—California. Morbidity and Mortality Weekly Report *28*(28):333-334.
21. Centers for Disease Control. 1980. Chagas' disease, Kalamazoo, Michigan. Morbidity and Mortality Weekly Report 20(13):147-8.
22. Centers for Disease Control. 1983. Viral hemorrhagic fever: Initial management of suspected and confirmed cases. Morbidity and Mortality Weekly Report (suppl) 32:25S-39S.
23. Centers for Disease Control. 1980. Smallpox Vaccines. Recommendation of the Immunization Practices Advisory Committee (ACIP). Morbidity and Mortality Weekly Report *29*:417-420.

24. Centers for Disease Control. 1981. Recommendations of the Immunization Practices Advisory Committee (ACIP) Diphtheria, Tetanus, and Pertussis. Morbidity and Mortality Weekly Report *30*(32):392-396.
25. Centers for Disease Control. I982. Recommendations of the Immunization Practices Advisory Committee (ACIP). Poliomyelitis prevention. Morbidity and Mortality Weekly Report *29*(3):22-26 and 31-34.
26. Centers for Disease Control. 1982. Plague vaccine. Selected recommendations of the Public Health Service Advisory Committee on Immunization Practices (ACIP). Morbidity and Mortality Weekly Report 31(22):301-304.
27. Centers for Disease Control. 1982. Recommendations of the Immunizations Practices Advisory Committee (ACIP). Inactivated hepatitis B virus vaccine. Morbidity and Mortality Weekly Report *31*(24):317-328.
28. Conant, N.F. 1955. Development of a method for immunizing man agains coccidioidomycosis, Third Quarterly Progress Report. Contract DA-18-064-CML-2563 Duke University, Durham, NC. Available from Defense Documents Center, AD 121-600.
29. Davidson, W.L. and Hummeler, K. 1960. B-virus infection in man. Ann NewYork Acad Sci *85* (article 3):970-979.
30. Denton, J.F., DiSalvo, A.F., Hirsch, M.L. 1967. Laboratory-acquired North American blastomycosis. JAMA *199*:935-936.
31. Dickson, E.C. 1937. Coccidioides infection: Part I. Arch Intern Med *59*:1029-1044.
32. Dickson, E.C. 1937. "Valley fever" of the San Joaquin Valley and fungus coccidioides. Calif Western Med *47*:151-155.
33. Dickson, E.C., Gifford, M.A. 1938. Coccidioides infection (coccidioidomycosis): II. The primary type of infection. Arch Intern Med *62*:853-871.
34. Diena, B.B., Wallace, R., Ashton, F.E., Johnson, W. and Patenaude, B. 1976. Gonococcal conjunctivitis: accidental infection. Can Med Assoc J *115*:609.
35. Donham, K.J. and Leninger, J.R. 1977. Spontaneous leprosy-like disease in a chimpanzee. J Infect Dis *136*:132-136.
36. Dowdle, W.R. and Hattwick, M.A.W. 1977. Swine influenza virus infections in humans. J Infect Dis *136*:Suppl:S386-389.
37. Edmond, R.T.D., Evans, B., Bowen, E.T.W. and Lloyd, G. 1977. A case of Ebola virus infection. Br Med J *2*:541-544.
38. Ellingson, H.V., Kadull, P.J., Bookwalter, H.L., Howe, C. 1946. Cutaneous anthrax: report of twenty-five cases. JAMA *131*:1105-8.
39. Evans, N. 1903. A clinical report of a case of blastomycosis of the skin from accidental inoculation. JAMA *40*:1172-1175.
40. Eyles, D.E., Coatney, G.R., and Getz, M.E. 1960. Vivax-type malaria parasite of macaques transmissible to man. Science *131*:1812-1813.
41. Fava, A. 1909. Un cas de sporotrichose conjonctivale et palpebrale primitives. Ann Ocul (Paris) *141*:338-343.
42. *Federal Register,* Vol. 41:27902-27943 1976. Recombinant DNA Research Guidelines.
43. *Federal Register,* Vol. 47:38048-38068 1982. Guidelines for Research Involving Recombinant DNA Molecules.
44. Fielitz, H. 1910. Ueber eine Laboratoriumsinfektion mit dem *Sporotrichum* de Beurmanni. Centralbl Bakteriol Parasitenk Abt I Orig 55:361-370.
45. Furcolow, M.L. 1961. Airborne Histoplasmosis. Bacteriol Rev *25*:301-309.
46. Gajdusek, D.C., Gibbs, C.J., Asher, D.M. Brown, P., Diwan, A., Hoffman, P., Nemo, G. Rohwer, R. and White, L. 1977. Precautions in the medical care and in handling materials from patients with transmissible virus dementia (Creutzfeldt-Jakob Disease). N Engl J Med *297*:1253-1258.
47. Good, R.C. and Snider, E.E., Jr., 1982. Isolation of nontuberculosis mycobacteria in the U.S. J Infect Dis *146*(6).
48. Green, R.N., Tuffnell, P.G. 1968. Laboratory-acquired melioidosis. Am J Med *44*:599-605.

49. Gutteridge, W.E., Cover, B., Cooke, A.J.D. 1974. Safety precautions for working with *Trypanosoma cruzi*. Trans R Soc Trop Med Hyg 68:161.
50. Halde, C. 1964. Percutaneous *Cryptococcus neoformans* inoculation without infection. Arch Dermatol *89*:545.
51. Hanel, E., Jr. and Kruse, R.H. 1967. Laboratory-acquired mycoses. Department of the Army, Miscellaneous Publication 28.
52. Hanson, R.P., et al. 1950. Human infections with the virus of vesicular stomatitis. J Lab Clin Med 36:754-758.
53. Hanson, R.P., Sulkin, S.E., Buescher, E.L., Hammond, W. McD., McKinney, R.W., and Work, T.E.: 1967. Arbovirus infections of laboratory workers. Science *158*, 1283-1286.
54. Harrell, E.R. 1964. The known and the unknown of the occupational mycoses, p. 176-178. *In* Occupational diseases acquired from animals. Continued Education Series No. 124, Univ Mich Sch Public Health, Ann Arbor, MI.
55. Harrington, J.M., and Shannon, H.S. 1976. Incidence of tuberculosis, hepatitis, brucellosis and shigellosis in British medical laboratory workers. Br Med J *1*, 759-762.
56. Hartley, E.G. 1966. "B" virus disease in monkeys and man. Br Vet J *122*:46-50.
57. Hattwick, M.A.W., O'Brien, R.J., and Hanson, B.F. 1976. Rocky Mountain Spotted Fever: epidemiology of an increasing problem. Ann Intern Med *84*:732-739.
58. Hennessen, W. 1971. Epidemiology of "Marburg Virus" disease. In: Martini, G.A., Siegert, R. eds., Marburg Virus Disease. New York: Springer-Verlag, pp. 161-165.
59. Huddleson, I.F. and Munger, M. 1940. A study of an epidemic of brucellosis due to *Brucella melitensis*. Am J Public Health *30*:944-954.
60. Jeanselme, E. and Chevallier, P. 1910. Chancres sporotrichosiques des doigts produits par la morsure d'un rat inocule de sporotrichose. Bull Mem Soc Med Hop (Paris) *30*:176-178.
61. Jeanselme, E. and Chevallier, P. 1911. Transmission de la sporotrichose a l'homme par les morsures d'un rat blanc inocule avec une nouvelle variete de *Sporotrichum*: Lymphangite gommeuse ascendante. Bull Mem Soc Med Hop (Paris) *31*:287-301.
62. Kaufmann, A.F. and Anderson, D.C. 1978. Tuberculosis control in nonhuman primates. In: Montali, R.J. (ed.). Mycobacterial Infections of Zoo Animals. Washington, D.C.: Smithsonian Institution Press, 227-234.
63. Kiley, M.P., Bowel, E.T.W., Eddy, G.A., Isaacson, M., Johnson, K.M., McCormick, J.B., Murphy, F.A., Pattyn, S.R., Peters, D., Prozesky, W., Regnery, R., Simpson, D.I.H., Slenczka, W., Sureau, P., van der Groen, G., Webb, P.A., and Sulff, H. 1982. Filoviridae: a taxonomic home for Marburg and Ebola viruses? Intervirology *18*:24-32.
64. Klutsch, K., Hummer, N., Braun, H., Heidland, A. 1965. Zur Klinik der Coccidioidomykose. Deut Med Wochensch *90*:1498-1501.
65. Kubica, G.P., Gross, Wm., Hawkins, J.E., Sommers, H.M., Vestal, A.L. and Wayne, L.G. 1975. Laboratory Services for Mycobacterial Diseases. Am Rev Respir Dis *112*:773-787.5.
66. Larsh, H. W. and Schwartz, J. 1977. Accidental inoculation-blastomycosis. Cutis *19*:334-336.
67. Leifer, E., Gocke, D.J., Bourne, H. 1970. Lassa fever, a new virus disease of man from West Africa. II. Report of a laboratory-acquired infection treated with plasma from a person recently recovered from the disease. Am J Trop Med Hyg *19*:677-9.
68. Looney, J.M., Stein, T. 1950. Coccidioidomycosis. N Engl J Med *242*:77-82.
69. Marchoux, P.E. 1934. Un cas d'inoculation accidentelle du bacille de Hanson en pays non lepreux. Int J Lepr *2*:1-7.
70. Martini, G.A., and Schmidt, H.A. 1968. Spermatogenic transmission of Marburg virus. Klin Wschr *46*:398-400.
71. McAleer, R. 1980. An epizootic in laboratory guinea pigs due to *Trichophyton mentagrophytes*. Aust Vet J *56*:234-236.
72. McDade, J.E. and Shepard, C.C. 1979. Virulent to avirulent conversion of Legionnaire's disease bacterium (*Legionella pneumophila*). Its effect on isolation techniques. J Infect Dis 139:707-711.

73. Melnick, J.L., Wenner, H.A., and Phillips, C.A. 1979. Enteroviruses. *In* Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th edition. Lennette, E.H. and Schmidt, N.J., Eds., Washington, D.C., American Public Health Association, pp. 471-534.
74. Meyer, K.F. 1915. The relationship of animal to human sporotrichosis: Studies on American sporotrichosis III. JAMA *65*:579-585.
75. Meyer, K.F., and Eddie, B.: 1941. Laboratory infections due to *Brucella*. J Infect Dis *68*:24-32.
76. Meyers, W.M., Walsh, G.P., Brown, H.L., Fukunishi, Y., Binford, C.H., Gerone, P.J. and Wolf, R.H. 1980. Naturally acquired leprosy in a mangabey monkey (*Cercocebus* sp.) Int J Lepr *48*:495-496.
77. Morisset, R., Spink, W.W. 1969. Epidemic canine brucellosis due to a new species, *Brucella canis*. Lancet 2:1000-2.
78. Murray, J.F. and Howard, D.H. 1964. Laboratory-acquired histoplasmosis. Am Rev Respir Dis *89*:631-640.
79. Nabarro, J.D.N. 1948. Primary pulmonary coccidioidomycosis: Case of laboratory infection in England. Lancet *1*:982-984.
80. National Sanitation Foundation Standard 49. 1983. Class II (Laminar Flow) Biohazard Cabinetry. Ann Arbor, MI.
81. Norden, A. 1951. Sporotrichosis: Clinical and laboratory features and a serologic study in experimental animals and humans. Acta Pathol Microbiol Scand. Suppl. *89*:3-119.
82. Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts. 1978. Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research. Bethesda, MD, National Institutes of Health.
83. Oliphant, J.W., and Parker, R.R.: 1948. Q fever: Three cases of laboratory infection. Public Health Rep *63*(42):1364-1370.
84. Oliphant, J.W., Gordon, D.A., Meis, A., and Parker, R.R.: 1949. Q fever in laundry workers, presumably transmitted from contaminated clothing. Am J Hyg *49*(1):76-82.
85. Oliver, J. and Reusser, T.R. 1942. Rapid method for the concentration of tubercle bacilli. Am Rev Tuberc *45*:450-452.
86. Oster, C.N., *et al*, 1977. Laboratory-acquired Rocky Mountain spotted fever. The hazard of aerosol transmission. N Engl J Med *297*:859-862.
87. Parritt, R.J. and Olsen, R.E. 1947. Two simultaneous cases of leprosy developing in tattoos. Am J Pathol *23*:805-817
88. Patterson, W.C., Mott, L.O., and Jenney, E.W. 1958. A study of vesicular stomatitis in man. J Am Vet Med Assoc 133(1):57-62.
89. Perkins, F.T. and Hartley, E.G. 1966. Precautions against B virus infection. Br Med J *1*:899-901.
90. Pike, R.M. 1976. Laboratory-associated infections: Summary and analysis of 3,921 cases. Health Lab Sci *13*:105-114.
91. Pike, R.M. 1978. Past and present hazards of working with infectious agents. Arch Pathol Lab Med *102*:333-336.
92. Pike, R.M. 1979. Laboratory-associated infections: incidence, fatalities, causes and prevention. Ann Rev Microbiol *33*:41-66.
93. Pike, R.M., Sulkin, S.E., and Schulze, M.L.: 1965. Continuing importance of laboratory-acquired infections. Am J Public Health *55*:190-199.
94. Prescott, J.F. and Karmali, M.A. 1978. Attempts to transmit *Campylobacter enteritis* to dogs and cats. Can Med Assoc J *119*:1001-1002.
95. Reid, D.D. 1957. Incidence of tuberculosis among workers in medical laboratories. Br Med J *2*:10-14.
96. Report of the Committee of Inquiry into the Smallpox Outbreak in London in March and April 1973. 1974. Her Majesty's Stationery Office, London.
97. Richardson, J. H.: 1973. Provisional summary of 109 laboratory-associated infections at the Center for Disease Control, 1947-1973. Presented at the 16th Annual Biosafety Conference, Ames, Iowa.

98. Riley, R.L. 1957. Aerial dissemination of pulmonary tuberculosis. Am Rev Tuberc *76*:931-941.
99. Riley, R.L. 1961. Airborne pulmonary tuberculosis. Bacteriol Rev *25*:243-248.
100. Robertson, D.H.H., Pickens, S., Lawson, J.H. and Lennex, B. 1980. An accidental laboratory infection with African trypanosomes of a defined stock. I and II. J Infect Dis *2*:105-112, 113-124.
101. Sastaw, S. and Carlisle, H.N. 1966. Aerosol infection of monkeys with *Rickettsia rickettsii*. Bacteriol Rev *30*:636-645.
102. Schlech, W.F., Turchik, J.B., Westlake, R.E., Klein, G.C., Band, J.D., Wever, R.E. 1981. Laboratory-acquired infection with *Pseudomonas pseudomallei* (melioidosis). N Engl J Med.:305:1133-1135.
103. Schwarz, J., Baum, G.L. 1951. Blastomycosis. Am J Clin Pathol *21*:999-1029.
104. Skinholj, P. 1974. Occupational risks in Danish clinical chemical laboratories. II. Infections. Scand J Clin Lab Invest *33*, 27-29.
105. Smith, C.E. 1950. The hazard of acquiring mycotic infections in the laboratory. Presented at 78th Ann. Meeting Am Public Health Assoc, St. Louis, MO.
106. Smith, C.E., Pappagianis, D., Levine, H.B. and Saito, M. 1961. Human coccidioidomycosis. Bacteriol Rev *25*:310-320.
107. Smith, D.T., Harrell, E.R., Jr. 1948. Fatal Coccidioidomycosis: A case of laboratory infection. Am Rev Tuberc *57*:368-374.
108. Smithwick, R.W. and Stratigos, C.B. 1978. Preparation of Acid-fast Microscopy Smears for Proficiency Testing and Quality Control. J Clin Microbiol *8*:110-111.
109. Spinelli, J.S., et al. 1981. Q fever crisis in San Francisco: Controlling a sheep zoonosis in a lab animal facility. Lab Anim *10*:(3):24-27.
110. Spink, W.W. 1956. *The Nature of Brucellosis*. Minneapolis, University of Minnesota Press, P. 106-8.
111. Sterne, M. and Wertzel, L.M. 1950. A new method of large-scale production of high-titer botulinum formol-toxoid types C and D. J Immunol *65*:175-183.
112. Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-borne Viruses. Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates. 1980. Am J Trop Med Hyg 29(6):1359-1381.
113. Sulkin, S.E., and Pike, R.M.: 1949. Viral Infections Contracted in the Laboratory. New Engl J Med *241*(5):205-213
114. Sulkin, S.E., and Pike, R.M.: 1951. Survey of laboratory-acquired infections. Am J Public Health *41*(7):769-781.
115. Sullivan, J.F., Songer, J.R., and Estrem, I.E.: 1978. Laboratory-acquired infections at the National Animal Disease Center, 1960-1976. Health Lab Sci *15*(1):58-64.
116. Tesh, R.B. and Schneidau, J.D., Jr. 1966. Primary cutaneous histoplasmosis. New Engl J Med *275*:597-599.
117. Thompson, D.W. and Kaplan, W. 1977. Laboratory-acquired sporotrichosis. Sabouraudia *15*:167-170.
118. Tomlinson, C.C., Bancroft, P. 1928. Granuloma coccidioides: Report of a case responding favorably to antimony and potassium tartrate. JAMA *91*:947-951.
119. Tosh, F.E., Balhuizen, J., Yates, J.L. and Brasher, C.A. 1964. Primary cutaneous histoplasmosis: Report of a case. Arch Intern Med *114*:118-119.
120. Walsh, G.P., Storrs, E.E., Burchfield, H.P. Cottrel, E.H., Vidrine, M.F., Binford, C.H. 1975. Leprosy-like disease occurring naturally in armadillos. J Reticuloendothel Soc *18*:347-351.
121. Wedum, A.G. and Kruse, R.H. 1969. Assessment of risk of human infection in the microbiology laboratory. Misc Pub 30, Industrial Health and Safety Directorate, Fort Detrick, Frederick, Md.
122. Wedum, A.G., Barkley, W.E., and Hellman, A. 1972. Handling of infectious agents. J Am Vet Med Assoc *161*:1557-1567.
123. Wedum, A.G.: History of Microbiological Safety: 1975. 18th Biological Safety Conference. Lexington, Kentucky.

124. Weissenbacher, M.C., Grela, M.E., Sabattini, M.S., Maiztegui, J.I., Coto, C.E., Frigerio, M.J., Cossio, P.M., Rabinovich, A.S., Oro, J.G.B. 1978. Inapparent infections with Junin virus among laboratory workers. J Infect Dis *137*:309-313.
125. Wilder, W. H. and McCullough, C.P. 1914. Sporotrichosis of the eye. JAMA *62*:1156-1160.
126. Willems, W.R., Kaluza, G., Boschek, C.B. and Bauer, H. 1979. Semliki Forest Virus: Cause of a fatal case of human encephalitis. Science *203*:1127-1129.
127. Wilson, J.W., Cawley, E.P., Weidman, F.D., and Gilmer, W.S. 1955. Primary cutaneous North American blastomycosis. Arch Dermatol *71*:39-45.
128. Wilson, J.W., Smith, C.E., Plunkett, O.A. 1953. Primary cutaneous coccidioidomycosis; the criteria for diagnosis and a report of a case. Calif Med *79*:233-239.
129. Winkler, W.G. 1973. Airborne rabies transmission in a laboratory worker. JAMA *226*(10):1219-1221.
130. World Health Organization. 1978. Smallpox surveillance. Weekly Epidemiological Record *53*(35):265-266.

Index

G

H

I

J

K

L

M

N

O

P

Y

Z

☆U.S. GOVERNMENT PRINTING OFFICE:1984 -746 -011/ 5277 REGION NO. 4