Section III—Principles of Biosafety

A fundamental objective of any biosafety program is the containment of potentially hazardous biological agents and toxins. The term containment describes a combination of primary and secondary barriers, facility practices and procedures, and other safety equipment, including personal protective equipment (PPE), for managing the risks associated with handling and storing hazardous biological agents and toxins in a laboratory environment. The purpose of containment is to reduce the risk of exposure to staff and the unintentional release of hazardous biological agents or toxins into the surrounding community and environment. Final determination on the combination of containment measures required to address the relevant biosafety risk present at a facility should be based on a comprehensive biosafety risk assessment. A comprehensive biosafety risk assessment is a key component of a successful biosafety program and should be part of an all-hazards risk assessment; it should be conducted on a continual basis to address evolving risks within the laboratory environment. Detailed information on the biological risk assessment process is found in Section II of BMBL.

Management and leadership, with support from the facility's biosafety professionals and other health and safety personnel, must perform and review the risk assessment using the best available information. Management and leadership are responsible for assessing the risks and selecting the appropriate combination of risk mitigation measures. All persons in the institution are responsible for performing their work in a manner that ensures the successful implementation and performance of the safety measures identified in the risk assessment and review.

Safety Equipment (Primary Barriers)

Primary barrier or primary containment is defined as physical containment measure(s) placed directly at the level of the hazard. Safety equipment such as biological safety cabinets (BSCs), enclosed containers, and other biosafety controls are designed to protect personnel, the surrounding community, and the environment from possible exposure to hazardous biological agents and toxins. Primary barriers can function to either provide containment (e.g., BSCs) or direct personal protection from the hazardous biological agents and toxins used. The BSC is the standard device used to provide containment of hazardous biological agents and toxins when conducting microbiological activities. Three primary types of BSCs (Class I, II, III) are used in laboratory facilities and selection of the appropriate BSC should be based on the risks identified for each respective laboratory. The three classes of BSCs are described and illustrated in Appendix A of BMBL.

Additional primary containment devices may include sealed containers (e.g., sealed rotors and centrifuge safety cups). These enclosed containers

are designed to contain aerosols, droplets, and leakage of hazardous biological agents and toxins that may result during certain activities (e.g., centrifugation). Sealed containers provide containment for transfers between laboratories within a facility, between facilities, and depending upon risk assessment, within a laboratory. Selection of the appropriate primary containment device should be based on the risks identified for those activities likely to produce aerosols, droplets, or result in potential leakage of hazardous biological agents and toxins.

Note that in some cases, such as when working with large animals, secondary barriers may become primary barriers. This lack of traditional primary barriers (e.g., BSC) can lead to additional risks to personnel, the surrounding community, and the environment. In these cases, the facility becomes the primary barrier and personnel must rely on administrative and personal protective equipment to reduce the risk of exposure. This type of facility may require additional enginerring controls and precautions (e.g., HEPA filtration on the exhaust air) to mitigate the risks posed to personnel, the surrounding community, and the environment.

Personal Protective Equipment

Personal protective equipment (PPE) helps protect the user's body from injury from a variety of sources (e.g., physical, electrical, heat, noise, chemical) or potential exposure to biological hazards and airborne particulate matter. PPE includes gloves, coats, gowns, shoe covers, closed-toe laboratory footwear, respirators, face shields, safety glasses, goggles, or ear plugs. PPE is usually used in combination with other biosafety controls (e.g., BSCs, centrifuge safety cups, and small animal caging systems) that contain the hazardous biological agents and toxins, animals, or materials being handled. In situations where a BSC cannot be used, PPE may become the primary barrier between personnel and the hazardous biological agents and toxins. Examples include fieldwork, resource-limited settings, certain animal studies, animal necropsy, and activities relating to operations, maintenance, service, or support of the laboratory facility. Selection of the appropriate PPE should be based on the risks identified for each respective laboratory.

Facility Design and Construction (Secondary Barriers)

The design and construction of the laboratory facility provide a means of secondary containment of hazardous biological agents and toxins. The secondary barriers, together with other biosafety controls, help provide protection of personnel, the surrounding community, and the environment from possible exposure to hazardous biological agents and toxins.

When the risk of infection by aerosol or droplet exposure is present, higher levels of secondary containment and multiple primary barriers may be used in combination with other controls to minimize the risk of exposure to personnel and the

unintentional release into the surrounding community or the environment. Such design features may include, but are not limited to the following:

- Ventilation strategies to ensure containment of the hazards;
- Effluent decontamination systems; and
- Specialized building/suite/laboratory configurations, including:
  - Controlled access zones to support the separation of the laboratory from office and public spaces;
  - Anterooms; and
  - Airlocks.

Design engineers may refer to specific ventilation recommendations as found in the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) Laboratory Design Guide.[1] Please note that depending on the laboratory facility, design professionals may need to follow or consult with the current versions of additional design recommendations and requirements such as:

- The National Institutes of Health (NIH) Design Requirements Manual (DRM);
- World Health Organization (WHO) Laboratory Biosafety Manual;
- World Organization for Animal Health (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals; and/or
- Other similar national or international design reference documents.

Facility Practices and Procedures

Established facility-specific best practices and procedures are essential to support the implementation and sustainability of a successful biosafety program. Persons working in facilities that handle and store hazardous biological agents and toxins must be able to properly identify all potential hazards and be trained and proficient in necessary safe practices and procedures. Management and leadership are responsible for providing and arranging the appropriate training of all personnel based on their functional roles and responsibilities in support of the biosafety program. Strict adherence to documented laboratory best practices and procedures is an essential element of a robust biosafety program since failure to follow the established procedures could result in an accidental exposure to personnel or unintentional release of hazardous biological agents and toxins into the surrounding community or the environment.

All facilities should develop and implement a biosafety program that identifies the hazards and specifies risk mitigation strategies to eliminate or reduce the likelihood of exposures and unintentional releases of hazardous materials. Management and leadership are ultimately responsible for the work conducted within laboratory facilities. When existing safety practices and procedures are not sufficient to minimize the risk(s) associated with a particular hazardous biological agent and/or toxin to an acceptable level, additional risk mitigation measures may

be needed. Safety best practices and procedures must be developed and implemented in coordination with other components of the overall biosafety program.

Biosafety Levels

The four primary Biosafety Levels (BSLs) for laboratories described in Section IV of BMBL consist of combinations of facility design features and safety equipment (primary and secondary barriers), facility practices and procedures, and personal protective equipment. Selection of the appropriate combinations to safely conduct the work should be based upon a comprehensive facility-specific biosafety risk assessment that documents the properties of the biological agents and toxins to be used, potential host characteristics, potential routes of infection, and the laboratory work practices and procedures conducted or anticipated to be used in the future. Recommended Biosafety Level(s) for the biological agents and toxins in Section VIII of BMBL represent suggested practices for work with an agent or toxin using standard protocols. Not all biological agents and toxins capable of causing disease in humans are included in Section VIII.

When working with well-defined organisms, identification of the appropriate biosafety controls should be based on the comprehensive biosafety risk assessment. However, when information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, an adjustment to the stringency of biosafety controls may be needed. For example, handling large volumes or high concentrations of a biological agent or toxin may require additional practices outlined in Sections IV and V of BMBL. Similarly, procedures that produce large amounts of aerosols may also require additional biosafety controls to reduce the likelihood of exposures to personnel and the unintentional release of a biological agent or toxin in the surrounding community or the environment. Furthermore, vaccines should not be considered non-pathogenic simply because they are vaccine strains.

It is important to note that the four Biosafety Levels described below are not to be confused and equated with Agent Risk Groups as described in the *National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).* The Risk Group (RG) of an agent is an important factor to be considered during the biosafety risk assessment process. Biological agents and toxins are assigned to their relevant Risk Groups based on their ability to cause disease in healthy human adults and spread within the community. However, just because a biological agent is listed as a Risk Group 3 agent, it does not mean the activities conducted with that biological agent must occur in a BSL-3 laboratory.

Biosafety Level 1

Biosafety Level 1 (BSL-1) standard practices, safety equipment, and facility specifications are generally appropriate for undergraduate and secondary educational training and teaching laboratories and for other laboratories that work with defined and characterized strains of viable biological agents not known to consistently cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberi*, infectious canine hepatitis virus, and exempt organisms under the *NIH Guidelines* are examples of the biological agents meeting these criteria. BSL-1 represents a basic level of containment that relies on standard, microbiological best practices and procedures with no special primary or secondary barriers, other than a door, a sink for handwashing, and non-porous work surfaces that are cleanable and easy to decontaminate.

Biosafety Level 2

Biosafety Level 2 (BSL-2) standard practices, safety equipment, and facility specifications are applicable to laboratories in which work is performed using a broad-spectrum of biological agents and toxins that are associated with causing disease in humans of varying severity. With good practices and procedures, these agents and toxins can generally be handled safely on an open bench, provided the potential for producing splashes and aerosols is low. Hepatitis B virus, human immunodeficiency virus (HIV), *Salmonella*, and *Toxoplasma* are examples of the biological agents that meet these criteria. Work done with any human, animal, or plant-derived specimens (e.g., blood, body fluids, tissues, or primary cell lines), where the presence of a biological agent or toxin may be unknown, can often be safely conducted under conditions typically associated with BSL-2.[3–5] Personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogens Standard for specific required precautions.[2]

The primary routes of exposure to personnel working with these types of biological agents and toxins relate to accidents including exposure via the percutaneous or mucosal routes and ingestion of potentially infectious materials. Extreme caution should be taken with contaminated needles and other sharp materials. Even though the biological agents and toxins routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential are conducted within primary containment equipment, such as a BSC or safety centrifuge cups. Furthermore, the use of primary containment equipment is also recommended when high-risk infectious agents are suspected to be present in any human, animal, or plant-derived specimens. Selection of the appropriate personal protective equipment should be based on the risks identified for each respective laboratory. Special practices for BSL-2 and ABSL-2 are recommended in Sections IV and V.

Secondary barriers should include those previously mentioned for BSL-1. Waste decontamination capabilities to reduce the potential of environmental contamination and the separation of laboratory spaces from office and public spaces to reduce the risk of exposure to other personnel should be considered.

Biosafety Level 3

Biosafety Level 3 (BSL-3) standard practices, safety equipment, and facility specifications are applicable to laboratories in which work is performed using indigenous or exotic biological agents with a potential for respiratory transmission and those that may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are examples of the biological agents that meet these criteria.

The primary routes of exposure to personnel working with these types of biological agents and toxins relate to accidental exposure via the percutaneous or mucosal routes and inhalation of potentially infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel, the surrounding community, and the environment from exposure to potentially infectious aerosols. All procedures involving the manipulation of infectious materials are conducted within a BSC or other primary containment device. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other primary containment strategies (e.g., centrifuge safety cups, sealed rotors or softwall containment enclosures) are implemented based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.

Secondary barriers for BSL-3 laboratories include those previously mentioned for BSL-1 and BSL-2 laboratories. They also include enhanced ventilation strategies to ensure inward directional airflow, controlled access zones to limit access to only laboratory approved personnel, and may contain anterooms, airlocks, exit showers, and/or exhaust HEPA filtration.

Biosafety Level 4

Biosafety Level 4 (BSL-4) standard practices, safety equipment, and facility specifications are applicable primarily for laboratories working with dangerous and exotic biological agents that pose a high individual risk of life-threatening disease that may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Marburg virus and Congo-Crimean hemorrhagic fever virus are examples of the biological agents that meet these criteria. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level or to re-designate the level.

The primary routes of exposure to personnel working with these types of biological agents relate to accidental exposure via the percutaneous and mucous membrane routes and inhalation of potentially infectious aerosols. The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a Class II BSC with a full-body, air-supplied positive-pressure personnel suit.

Secondary barriers for BSL-4 laboratories should include those previously mentioned for previous Biosafety Levels. Additionally, the BSL-4 facility itself is often a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems, for both solid and liquid waste, to prevent the release of hazardous biological agents into the surrounding community and the environment.

Animal Facilities

Four primary Biosafety Levels are also described for activities involving hazardous biological agent and toxin work conducted with animals. These four combinations of facility design and construction, safety equipment, and practices and procedures are designated Animal Biosafety Levels (ABSL) 1, 2, 3, and 4, and provide increasing levels of protection to personnel, the surrounding community, and the environment.

One additional Biosafety Level, designated Animal Biosafety Level 3-Agriculture (ABSL-3Ag) addresses activities involving the use of hazardous biological agents and toxins designated as High-Consequence Foreign Animal Diseases and Pests by the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) in large or loose-housed animals. ABSL-3Ag laboratories are designed so that the laboratory building itself serves as a primary barrier to prevent the unintentional release of these high consequence agents into the environment. More information on the design and operation of ABSL-3Ag facilities and USDA/APHIS High-Consequence Foreign Animal Diseases and Pests is provided in Appendix D of BMBL. Appendix D also provides guidance for containment of loose-housed or open penned animals at other containment levels, designated ABSL-2Ag and ABSL-4Ag.

Clinical Laboratories

Clinical laboratories routinely work with unknown specimens and specimens that have the potential to be infected with multiple pathogens; as such, the occupational risks in a clinical laboratory environment differ from those of a research or teaching laboratory. Most public and animal health clinical laboratories use Biosafety Level 2 (BSL-2) facility, engineering, and biosafety practices.[5] Clinical diagnostic laboratory personnel may not know what infectious agent or other hazard(s) exist in the specimen they handle and process. More information on clinical laboratory biosafety is provided in Appendix N.

Laboratory Biosecurity

In recent years, with the passing of federal legislation regulating the possession, use, and transfer of biological Select Agents and Toxins with high adverse public health and/or agricultural consequences (DHHS, USDA APHIS Select Agents), a much greater emphasis has been placed in the emerging field of biosecurity. Biosecurity and Select Agent issues are covered in detail in Section VI and Appendix F of BMBL. While biosafety focuses on the protection of personnel, the surrounding community, and the environment from the unintentional release of hazardous biological agents and toxins, the field of laboratory biosecurity is focused on the prevention of the theft, loss, and misuse of hazardous biological agents and toxins, equipment, and/or valuable information by an individual(s) for malicious use. Nonetheless, a successful containment strategy must incorporate aspects of both biosafety and laboratory biosecurity to adequately address the risks present at the facility.

References

1. American Society of Heating, Refrigerating and Air-Conditioning Engineers. ASHRAE Laboratory Design Guide: Planning and Operation of Laboratory HVAC Systems. 2nd ed. Atlanta (GA): ASHRAE; 2015.
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3. Centers for Disease Control and Prevention. Update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. MMWR Morb Mortal Wkly Rep. 1988;37(24):377–82, 387–8.
4. Garner JS. Guideline for isolation precautions in hospitals. The Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol. 1996;17(1):53–80. Erratum in: Infect Control Hosp Epidemiol. 1996;17(4):214.
5. CLSI. Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Fourth Edition. CLSI document M29-A4. Wayne (PA): Clinical and Laboratory Standards Institute; 2014.

Section IV—Laboratory Biosafety Level Criteria

The essential elements of the Biosafety Levels 1–4 are standard microbiological practices, special practices, safety equipment, and laboratory facilities as discussed in Section III; these elements apply to activities involving infectious microorganisms, toxins, and laboratory animals. The four levels are organized in ascending order by the degree of protection provided to personnel, the environment, and the community. Special practices address any unique risks associated with the handling of agents requiring increasing levels of containment. Appropriate safety equipment and laboratory facilities enhance worker and environmental protection.

The features of each Biosafety Level (BSL) are summarized in Table 1 of this section. Adjustments to the containment levels described are based on an assessment of all risks, as detailed in Section II. Each facility ensures that worker safety and health concerns are coordinated with the Institutional Biosafety Committee (IBC), or equivalent resource, and/or other applicable institutional safety committee(s) and that all hazards are addressed as part of the protocol review process. Additional occupational health information is provided in Section VII.

Biosafety Level 1

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

The following standard practices, safety equipment, and facility specifications are recommended for BSL-1.

A. Standard Microbiological Practices

1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained.

Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.

3. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. See Section VII.

4. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.

    a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.

    b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

5. A sign is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.

6. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.

7. Gloves are worn to protect hands from exposure to hazardous materials.
    a. Glove selection is based on an appropriate risk assessment.
    b. Gloves are not worn outside the laboratory.
    c. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    d. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.
8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
9. Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.
10. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
11. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
12. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
    a. Plasticware is substituted for glassware whenever possible.
    b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        i. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        ii. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        iii. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle

(e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).

iv. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.

c. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

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

14. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.

15. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:

    a. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.

    b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.

16. An effective integrated pest management program is implemented. See Appendix G.

17. Animals and plants not associated with the work being performed are not permitted in the laboratory.

B. Special Practices

None required.

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

1. Special containment devices or equipment, such as biosafety cabinets (BSCs), are not generally required.
2. Protective laboratory coats, gowns, or uniforms are worn to prevent contamination of personal clothing.
3. Protective eyewear is worn by personnel when conducting procedures that have the potential to create splashes and sprays of microorganisms or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories have doors for access control.
2. Laboratories have a sink for handwashing.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
    a. Carpets and rugs in laboratories are not appropriate.
    b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Laboratory furniture can support anticipated loads and uses.
    a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
6. Laboratory windows that open to the exterior are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

Biosafety Level 2

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

The following standard and special practices, safety equipment, and facility specifications are recommended for BSL-2.

A. Standard Microbiological Practices

1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
3. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. See Section VII.
4. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.

a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.

b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

5. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
6. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
7. Gloves are worn to protect hands from exposure to hazardous materials.
   a. Glove selection is based on an appropriate risk assessment.
   b. Gloves are not worn outside the laboratory.
   c. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
   d. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.
8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
9. Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.
10. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
11. Mouth pipetting is prohibited. Mechanical pipetting devices are used.

12. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
    a. Plasticware is substituted for glassware whenever possible.
    b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        i. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        ii. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        iii. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
        iv. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
    c. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
13. Perform all procedures to minimize the creation of splashes and/or aerosols.

14. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
15. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
16. An effective integrated pest management program is implemented. See Appendix G.
17. Animals and plants not associated with the work being performed are not permitted in the laboratory.

B. Special Practices

1. Access to the laboratory is controlled when work is being conducted.
2. The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-2 containment.
3. Laboratory personnel are provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
4. Properly maintained BSCs or other physical containment devices are used, when possible, whenever:
    a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotors or centrifuge safety cups with loading and unloading of the rotors and centrifuge safety cups in the BSC or another containment device.

c. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment and administrative controls are used, based on a risk assessment.

5. Laboratory equipment is decontaminated routinely; after spills, splashes, or other potential contamination; and before repair, maintenance, or removal from the laboratory.

6. A method for decontaminating all laboratory waste is available (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

7. Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the laboratory supervisor and any other personnel designated by the institution. Appropriate records are maintained.

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

1. Protective laboratory coats, gowns, or uniforms designated for laboratory use are worn while working with hazardous materials and removed before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). Protective clothing is disposed of appropriately or deposited for laundering by the institution. Laboratory clothing is not taken home.

2. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.

3. The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, relevant staff are enrolled in a properly constituted respiratory protection program.

4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors are self-closing and have locks in accordance with the institutional policies.
2. Laboratories have a sink for handwashing. It should be located near the exit door.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
    a. Carpets and rugs in laboratories are not appropriate.
    b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Laboratory furniture can support anticipated loads and uses.
    a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
6. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. See Appendix A, Figure 11. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment.
9. There are no specific requirements for ventilation systems. However, the planning of new facilities considers mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
    a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

b. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.

c. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.

Biosafety Level 3

Biosafety Level 3 (BSL-3) is suitable for work with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel receive specific training in handling pathogenic and potentially lethal agents, and they are supervised by scientists competent in handling infectious agents and associated procedures.

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

The following standard and special practices, safety equipment, and facility specifications are recommended for BSL-3.

A. Standard Microbiological Practices

1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
3. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having

such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. See Section VII.

4. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
    a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
5. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
6. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
7. Gloves are worn to protect hands from exposure to hazardous materials.
    a. Glove selection is based on an appropriate risk assessment.
    b. Gloves are not worn outside the laboratory.
    c. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    d. Do not wash or reuse disposable gloves and dispose of used gloves with other contaminated laboratory waste.
8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.

9. Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.
10. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
11. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
12. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
    a. Plasticware is substituted for glassware whenever possible.
    b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        i. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        ii. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        iii. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
        iv. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
    c. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

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

14. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.

15. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:

    a. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.

    b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.

16. An effective integrated pest management program is implemented. See Appendix G.

17. Animals and plants not associated with the work being performed are not permitted in the laboratory.

B. Special Practices

1. All persons entering the laboratory are advised of the potential hazards and meet specific entry/exit requirements in accordance with institutional policies. Only persons whose presence in the facility or laboratory areas is required for scientific or support purposes are authorized to enter.

2. All persons who enter operational laboratory areas are provided information on signs and symptoms of disease and receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.

3. The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-3 containment.
4. A system is established for reporting and documenting near misses, laboratory accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
5. Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the laboratory supervisor, institutional management, and appropriate safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.
6. Biological materials that require BSL-3 containment are placed in a durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the laboratory. Once removed, the primary container is opened within a BSC in BSL-3 containment unless a validated inactivation method is used. See Appendix K. The inactivation method is documented in-house with viability testing data to support the method.
7. All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. No work with open vessels is conducted on the bench. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of personal protective equipment and other administrative and/or engineering controls, such as centrifuge safety cups or sealed rotors, are used, based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.
8. Laboratory equipment is routinely decontaminated after spills, splashes, or other potential contamination, and before repair, maintenance, or removal from the laboratory.
    a. Equipment or material that might be damaged by high temperatures or steam is decontaminated using an effective and verified method, such as a gaseous or vapor method.
9. A method for decontaminating all laboratory waste is available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

10. Decontamination of the entire laboratory is considered when there has been gross contamination of the space, significant changes in laboratory usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory is based on a risk assessment.
11. Decontamination processes are verified on a routine basis.

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

1. Laboratory workers wear protective clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
2. Based on work being performed, additional PPE may be required.
    a. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splash guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
    b. Two pairs of gloves are worn when appropriate.
    c. Respiratory protection is considered. Staff wearing respiratory protection are enrolled in a properly constituted respiratory protection program.
    d. Shoe covers are considered.
3. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

D. Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building.
    a. Laboratory access is restricted. Laboratory doors are lockable in accordance with institutional policies. Access to the laboratory is through two consecutive self-closing doors. A clothing change room and/or an anteroom may be included in the passageway between the two self-closing doors.
2. Laboratories have a sink for handwashing. The sink is hands-free or automatically operated and should be located near the exit door.

If a laboratory suite is segregated into different zones, a sink is also available for handwashing in each zone.

3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping.
    a. Carpets and rugs are not permitted.
    b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
    c. Seams, floors, walls, and ceiling surfaces are sealed. Spaces around doors and ventilation openings are capable of being sealed to facilitate space decontamination.
    d. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases.
    e. Walls and ceilings are constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
5. Laboratory furniture can support anticipated loads and uses.
    a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant.
6. All windows in the laboratory are sealed.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. See Appendix A, Figure 11. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment. Vacuum lines not protected as described are capped. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.
9. A ducted mechanical air ventilation system is required. This system provides sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory is designed such that under failure conditions the airflow will not be reversed at the containment barrier.

a. A visual monitoring device that confirms directional airflow is provided at the laboratory entry. Audible alarms to notify personnel of airflow disruption are considered.

b. The laboratory exhaust air is not re-circulated to any other area in the building.

c. The laboratory exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA filtered.

10. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.

    a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

    b. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.

    c. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.

    d. Class III BSCs are provided supply air in such a manner that prevents positive pressurization of the cabinet or the room.

11. Equipment that may produce infectious aerosols is used within primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters are tested annually and replaced as needed.

12. Facility is constructed to allow decontamination of the entire laboratory when there has been gross contamination of the space, significant changes in usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory is based on the risk assessment.

    a. Facility design consideration is given to means of decontaminating large pieces of equipment before removal from the laboratory.

13. Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These laboratory enhancements may include one or more of the following: an anteroom for clean storage of equipment and supplies

with dress-in, shower-out capabilities; gas-tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; containment of other piped services; or advanced access control devices, such as biometrics.

14. When present, HEPA filter housings have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. All HEPA filters are located as near as practicable to the laboratory to minimize the length of potentially contaminated ductwork. The HEPA filter housings allow for leak testing of each filter and assembly. The filters and housings are certified at least annually.
15. The BSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified as necessary by operational experience. The verification code for this document is 609588
16. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress are developed and implemented.

Biosafety Level 4

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

There are two models for BSL-4 laboratories:

1. Cabinet Laboratory: manipulation of agents is performed in a Class III BSC; and
2. Suit Laboratory: personnel wear a positive-pressure supplied-air protective suit.

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

The following standard and special practices, safety equipment, and facility specifications are necessary for BSL-4.

A. Standard Microbiological Practices

1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
3. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. See Section VII.
4. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
    a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility

malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

5. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
6. Long hair is restrained so that it cannot contact hands, specimen, containers, or equipment
7. Gloves are worn to protect hands from exposure to hazardous materials.
    a. Glove selection is based on an appropriate risk assessment.
    b. Inner gloves are not worn outside the laboratory.
    c. Change inner gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    d. Do not wash or reuse disposable gloves,and dispose of used gloves with other contaminated laboratory waste.
8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
9. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
10. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
    a. Plasticware is substituted for glassware whenever possible.

b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.

    i. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.

    ii. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

    iii. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).

    iv. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.

c. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

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

13. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.

14. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. A method for decontaminating all laboratory wastes is available in the laboratory

(e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). See B. Special Practices, #7 in the following sub-section for additional details.

15. An effective integrated pest management program is implemented. See Appendix G.
16. Animals and plants not associated with the work being performed are not permitted in the laboratory.

B. Special Practices

1. All persons entering the laboratory are advised of the potential hazards and meet specific entry/exit requirements in accordance with institutional policies. Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes are authorized to enter. Additional training/security requirements may be required prior to gaining independent access to BSL-4 laboratories.
2. All persons who enter operational laboratory areas are provided information on signs and symptoms of disease and receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
    a. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known Laboratory-associated infections.
3. Laboratory personnel and support staff are trained and approved to work in the facility. The laboratory supervisor is responsible for ensuring that, prior to working independently with agents requiring BSL-4 containment, laboratory personnel demonstrate high proficiency in standard and special microbiological practices and techniques for working with agents requiring BSL-4 containment. Personnel are required to read and follow instructions on practices, and procedural changes are addressed as part of the protocol review.
4. A system is established for reporting and documenting near misses, laboratory accidents, exposures, unanticipated absence due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
5. Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the laboratory supervisor, institutional management, and appropriate safety,

compliance, and security personnel according to institutional policy. Appropriate records are maintained.

6. Biological materials that require BSL-4 containment are placed in a durable, leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the BSL-4 facility by authorized personnel. These materials are transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower for receipt by authorized personnel. Once removed, the primary container is not to be opened outside BSL-4 containment unless a validated inactivation method is used (e.g., gamma irradiation). See Appendix K. The inactivation method is documented in-house with viability testing data to support the method.
7. All waste is decontaminated by a verified method prior to removal from the laboratory.
8. Equipment is routinely decontaminated and is decontaminated after spills, splashes, or other potential contamination and before repair, maintenance, or removal from the laboratory.
    a. Equipment or material that might be damaged by high temperatures or steam is decontaminated using an effective and verified method, such as a gaseous or vapor method, in an airlock or chamber designed for this purpose.
9. A logbook, or other means of documenting the date and time of all persons entering and leaving the laboratory, is maintained.
10. An inventory system for agents stored within the laboratory is in place.
11. While the laboratory is operational, personnel enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing and jewelry (except eyeglasses) are removed in the outer clothing change room. All persons entering the laboratory use laboratory clothing, including undergarments, pants, shirts, socks, jumpsuits, shoes, and gloves, as appropriate. All persons leaving the laboratory take a personal body shower. Used laboratory clothing and other waste, including gloves, are not removed from the inner change room through the personal shower. These items are treated as contaminated materials and decontaminated before laundering or disposal.
12. After the laboratory has been completely decontaminated by verification of a validated method and all infectious agents are secured, necessary staff may enter and exit without following the clothing change and shower requirements described above.

13. Daily inspections of essential containment and life support systems are completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.

14. Only necessary equipment and supplies are stored inside the laboratory. All equipment and supplies taken inside the laboratory are decontaminated before removal from the laboratory.

    a. Supplies and materials that are not brought into the laboratory through the change room are brought in through a dunk tank, previously decontaminated double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. The inner door is secured after materials are brought into the facility. The outer door of the autoclave or fumigation chamber is not opened until the autoclave, fumigation chamber, or airlock has been operated through a successful decontamination cycle.

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

Cabinet Laboratory

1. All procedures involving the manipulation of infectious materials are conducted within a Class III BSC.

2. A Class III BSC contains:

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

    b. A pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment between the cabinet and the surrounding laboratory is maintained at all times.

    c. A HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. Supply air is provided in such a manner that prevents positive pressurization of the cabinet. There are gas-tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium are present on all HEPA filter housings.

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

e. Gloves that are inspected for damage prior to use and changed if necessary. Gloves are replaced annually during cabinet recertification.

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

4. Manipulation of high concentrations or large volumes of infectious agents within the Class III BSC is performed using physical containment devices inside the cabinet whenever practical. Such materials are centrifuged inside the cabinet using sealed rotors or centrifuge safety cups.

5. The interior of the Class III BSC and all contaminated plenums, fans, and filters are decontaminated using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shutdowns, and in other situations, as determined by risk assessment. Success of the decontamination is verified before accessing the interior spaces of the cabinet.

6. The Class III BSC is certified at least annually.

7. For Class III BSCs directly connected via a double-door, pass-through to a BSL-4 suit laboratory, materials may be placed into and removed from the Class III BSC via the suit laboratory.

8. Workers in the laboratory wear protective laboratory clothing with a solid front, such as tie-back or wrap-around gowns, scrubs, or coveralls. Shoe coverings are considered based on a risk assessment.

    a. Upon exit, all protective clothing is removed in the inner change room before showering.

    b. Prescription eyeglasses are decontaminated before removal through the personal body shower.

9. Disposable gloves are worn underneath cabinet gloves to protect the worker from exposure should a break or tear occur in a cabinet glove.

Suit Laboratory

1. All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment devices. No work with open vessels is conducted on the bench.
2. Equipment that may produce aerosols is used within primary barrier devices that exhaust air through HEPA filtration before being discharged into the laboratory or facility exhaust system. These HEPA filters are tested annually and replaced as needed.
3. Materials centrifuged in the laboratory use sealed rotors or centrifuge safety cups. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.
4. All procedures are conducted by personnel wearing a one-piece, positive-pressure supplied-air suit.
    a. All persons don laboratory clothing, such as scrubs, before entering the room used for donning positive-pressure suits.
    b. Procedures are in place to control and verify the operation of the one-piece positive-pressure supplied-air suit, including gloves, before each use.
    c. Decontamination of outer suit gloves is performed during the course of normal laboratory operations to remove gross contamination and minimize further contamination of the laboratory.
    d. Inner disposable gloves are worn to protect the laboratorian should a break or tear in the outer suit gloves occur. Disposable inner gloves are not worn outside the inner change area.
    e. Upon exit from the chemical shower, inner gloves and all laboratory clothing are removed and discarded or collected for autoclaving before laundering prior to entering the personal shower.
    f. Prescription eyeglasses are decontaminated before removal through the personal body shower.

D. Laboratory Facilities (Secondary Barriers)

Cabinet Laboratory

1. The BSL-4 cabinet facility may be located in a separate building or a clearly demarcated and isolated zone within a building.
    a. Facility access is restricted. Laboratory doors are lockable.

b. Exit from the laboratory is by sequential passage through an inner (i.e., dirty) changing area, a personal shower, and an outer (i.e., clean) change room upon exiting the cabinet laboratory.

2. An automatically activated emergency power source is provided, at a minimum, for the laboratory exhaust system, alarms, lighting, entry and exit controls, BSCs, and door gaskets.

    a. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems are on an uninterrupted power supply (UPS).

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

4. A hands-free sink is provided near the door of the cabinet laboratory(ies) and the inner change room. A sink is provided in the outer change room.

5. An eyewash station is readily available in the laboratory.

6. Walls, floors, and ceilings of the cabinet laboratory are constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell are resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors are monolithic, sealed, and coved.

    a. All penetrations in the internal shell of the cabinet laboratory and inner change room are sealed.

    b. Openings around doors into the cabinet laboratory and inner change room are minimized and capable of being sealed to facilitate decontamination.

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

8. Furniture is minimized, of simple construction, and capable of supporting anticipated loads and uses.

    a. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination.

    b. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

c. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated.

9. Windows are break-resistant and sealed.
10. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
11. If Class II BSCs or other primary containment barrier systems are needed in the cabinet laboratory, they are installed and operated in a manner to ensure their effectiveness. See Appendix A.

    a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

    b. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Cabinet exhaust air passes through two HEPA filters, including the HEPA in the BSC, prior to release outside. Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.

    c. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.

12. Central vacuum systems are discouraged. If there is a central vacuum system, it does not serve areas outside the cabinet. Two in-line HEPA filters are placed near each use point and overflow collection is provided while in use. Filters are installed to permit in-place decontamination and replacement.
13. A dedicated, non-recirculating ventilation system is provided. Only cabinet laboratories with the same HVAC requirements (i.e., other BSL-4 cabinet laboratories, ABSL-4 cabinet facilities) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

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

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

c. The ventilation system is monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device is installed outside of containment so proper differential pressures within the laboratory may be verified prior to entry and during regular checklist procedures. Visual monitoring is also in place within containment.

d. Supply air to and exhaust air from the cabinet laboratory, inner change room, and fumigation/decontamination chambers pass through a HEPA filter. The air exhaust discharge is located away from occupied spaces and building air intakes.

e. All HEPA filters are located as near as practicable to the cabinet and laboratory to minimize the length of potentially contaminated ductwork. All HEPA filters are tested and certified annually.

f. The HEPA filter housings are designed to allow for in situ decontamination and verification of the validated decontamination process prior to removal. The design of the HEPA filter housing has gas-tight isolation dampers, decontamination ports, and the ability to individually scan each filter in the assembly for leaks.

14. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet laboratory(ies). Access to the exit side of the pass-through is limited to those with authorized access to the BSL-4 laboratory and with specific clearance, if required.

15. Liquid effluents from cabinet laboratory sinks, floor drains, autoclave chambers, and other sources within the cabinet laboratory are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

    a. Decontamination of all liquid effluents is documented. The decontamination process for liquid effluents is validated physically and biologically. Biological validation is performed at least annually or more often, if required by institutional policy.

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

16. A double-door, pass-through autoclave is provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory are sealed to the wall through which the autoclave passes. This bioseal is durable, airtight, and capable of expansion and contraction. Positioning the bioseal so that the equipment

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

   a. Gas discharge from the autoclave chamber is HEPA-filtered or decontaminated. Autoclave decontamination processes are designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

17. The facility design parameters and operational procedures are documented. The facility is tested to verify that the design and operational parameters have been met prior to operation. Facilities are also re-tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified, as necessary, by operational experience.

18. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, video, and computer). Provisions for emergency communication and emergency access or egress are developed and implemented.

Suit Laboratory

1. The BSL-4 suit facility may be located in a separate building or a clearly demarcated and isolated zone within a building.

   a. Facility access is restricted. Laboratory doors are lockable.

   b. Entry into the laboratory is through an airlock fitted with airtight doors.

   c. Exit from the laboratory is by sequential passage through the chemical shower, inner (i.e., dirty) change room, personal shower, and outer (i.e., clean) changing area.

2. Personnel who enter this area wear a positive-pressure suit supplied with HEPA-filtered breathing air. The breathing air systems have redundant compressors, failure alarms, and emergency back-up capable of supporting all workers within the laboratory to allow the personnel to safely exit the laboratory.

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

4. An automatically activated emergency power source is provided at a minimum for the laboratory exhaust system, alarms, lighting, entry and exit controls, BSCs, and door gaskets.
    a. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems are on an uninterrupted power supply (UPS).
5. A double-door autoclave, dunk tank, or fumigation chamber is provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory.
6. Hands-free sinks inside the suit laboratory are placed near procedure areas.
7. An eyewash station for use during maintenance is readily available in the laboratory area.
8. Walls, floors, and ceilings of the laboratory are constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell are resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors are monolithic, sealed, and coved.
    a. All penetrations in the internal shell of the laboratory, suit storage room, and the inner change room are sealed.
9. Services and plumbing that penetrate the laboratory walls, floors, or ceiling are installed to ensure that no backflow from the laboratory occurs. Breathing air systems are exempt from this provision. These penetrations are fitted with two (in series) backflow prevention devices. Consideration is given to locating these devices outside of containment. Atmospheric venting systems are provided with two HEPA filters in series and are sealed up to the second filter.
10. Decontamination of the entire laboratory is performed using a validated gaseous or vapor method when there have been significant changes in usage, before major renovations or maintenance shutdowns, and in other situations, as determined by risk assessment. Decontamination is verified prior to any change in the status of the laboratory.
11. Furniture is minimized, of simple construction, and capable of supporting anticipated loads and uses.
    a. Spaces between benches, cabinets, and equipment are accessible for cleaning, decontamination, and unencumbered movement of personnel.

b. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

c. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated.

d. Sharp edges and corners are avoided.

12. Windows are break-resistant and sealed.

13. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

14. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.

    a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

    b. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III), which contains a HEPA filter.

    c. Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet. The unique code for this document is 609588

    d. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.

    e. Class III BSCs are provided supply air in such a manner that prevents positive pressurization of the cabinet or the room.

15. Central vacuum systems are discouraged. If there is a central vacuum system, it does not serve areas outside the laboratory. Two in-line HEPA filters are placed near each use point and overflow collection is provided while in use. Filters are installed to permit in-place decontamination and replacement. Consideration is made to the provision of two HEPA filters in series as close to the vacuum pump as possible.

16. A dedicated, non-recirculating ventilation system is provided. Only laboratories or facilities with the same HVAC requirements (i.e., other BSL-4 laboratories, ABSL-4, ABSL-3Ag, ABSL-4Ag facilities) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

a. The ventilation system is designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory.

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

c. The ventilation system is monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device is installed outside of containment so proper differential pressures within the laboratory may be verified prior to entry and during regular checklist procedures. Visual monitoring is also in place within containment.

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

e. All HEPA filters are located as near as practicable to the laboratory to minimize the length of potentially contaminated ductwork. All HEPA filters are tested and certified annually.

f. The HEPA filter housings are designed to allow for in situ decontamination of the filter and verification of the validated process prior to removal. The design of the HEPA filter housing has gas-tight isolation dampers, decontamination ports, and the ability to individually scan each filter in the assembly for leaks.

17. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the laboratory. Access to the exit side of the pass-through is limited to those individuals authorized to be in the facility and provided appropriate clearance if required.

18. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

    a. Decontamination of all liquid effluents is documented. The decontamination process for liquid effluents is validated physically and

biologically. Biological validation is performed at least annually or more often if required by institutional policy.

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

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

    a. Gas discharge from the autoclave chamber is HEPA-filtered or is decontaminated. Autoclave decontamination processes are designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

20. The facility design parameters and operational procedures are documented. The facility is tested to verify that the design and operational parameters have been met prior to operation. Facilities are also re-tested annually or after significant modification to ensure operational parameters are maintained. Verification criteria are modified, as necessary, by operational experience.

21. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, video, and computer). Provisions for emergency communication and emergency access or egress are developed and implemented.

Table 1. Summary of Laboratory Biosafety Levels (BSLs)

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory

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BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls;[b] gloves;[b] full-body, air-supplied, positive-pressure suit[c]	Entry sequence; entry through airlock with airtight doors;[c] walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system required; double-door, pass-through autoclave required

a. Each successive BSL contains the recommendations of the preceding level(s) and the criteria in the cell.
b. Applies to Cabinet Laboratory
c. Applies to Suit Laboratory