

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

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivarium research facilities) and applies to the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, institutional management provides facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security, and care for the laboratory animal.<sup>1</sup> Laboratory animal facilities are to be considered a special type of laboratory. As a general principle, the Biosafety Level (e.g., facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

The animal room can present unique concerns. Animals may generate aerosols, may bite and scratch, and/or may be infected with a zoonotic agent. The application of the Animal Biosafety Levels (ABSL) is determined by a protocol-driven risk assessment.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care are approved by an Institutional Animal Care and Use Committee (IACUC)<sup>2</sup> and meet applicable standards and regulations (e.g., *Guide for the Care and Use of Laboratory Animals*,<sup>3</sup> Animal Welfare Regulations).<sup>4,5</sup> In addition, the organization has an occupational health and safety program that addresses potential hazards associated with the conduct of laboratory animal research. *Occupational Health and Safety in the Care and Use of Research Animals*,<sup>6</sup> published by the Institute for Laboratory Animal Research (ILAR), is most helpful in this regard. Additional safety guidance on working with non-human primates (NHPs) is available in the ILAR publication, *Occupational Health and Safety in the Care and Use of Nonhuman Primates*.<sup>7</sup>

Personnel receive specific training in humane animal care and handling in accordance with the appropriate regulatory requirements and guidance documents (e.g., Animal Welfare Regulations,<sup>4</sup> *Guide for the Care and Use of Laboratory Animals*,<sup>3</sup> and taxon-specific publications for wild/exotic animals) as well as animal facility procedures, and are supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures. This includes training on proper use of engineering controls, including biosafety cabinets (BSCs) or downdraft tables, as well as personal protective equipment (PPE) appropriate to the ABSL as determined by a risk assessment. The biosafety officer (BSO), the IBC, or equivalent resource, and/or other applicable committees are responsible for the review of protocols and policies to protect personnel who manipulate and care for animals from hazardous exposures.

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

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These four combinations, designated ABSL-1–4, provide increasing levels of protection to personnel and to the environment, and are recommended as minimum standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents requiring BSL-1–4 containment, respectively. Investigators who are inexperienced should seek help in designing their experiments from individuals experienced in this specialized work.

In addition to the ABSLs described in this section, the USDA has developed facility parameters and work practices for handling agents of agricultural significance. [Appendix D](#) includes a discussion on Animal Biosafety Levels 2, 3, and 4 Agriculture (ABSL-2Ag, ABSL-3Ag, ABSL-4Ag). The “Ag” designation is used for animals that are loose-housed or in open penning and may be exposed to agents of concern from an agricultural perspective. USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. [Appendix D](#) also describes some of the enhancements beyond standard recommendation at ABSL-2–4 that may be required by USDA APHIS when working in the laboratory or vivarium with certain veterinary agents of concern.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in this section. Please refer to [Appendix E](#) for additional information on the Arthropod Containment Guidelines (ACG).

### **Animal Biosafety Level 1**

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

Special containment equipment or facility design may be required as determined by risk assessment. See [Section II](#) for additional information on the Biological Risk Assessment.

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

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

**A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as well as the Institutional Biosafety Committee (IBC), as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); 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.
5. Personal health status may affect an individual's susceptibility to infection or 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](#). Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.

6. Appropriate occupational medical services are in place, as determined by risk assessment.
  - a. An animal allergy prevention program is part of the medical surveillance.
  - b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. 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 experimental animals, 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, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the room's Animal 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 animal room. Agent information is posted in accordance with the institutional policy.
9. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.

10. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - a. Glove selection is based on an appropriate risk assessment.<sup>8-12</sup>
  - b. Consider the need for bite and/or scratch-resistant gloves.
  - c. Gloves worn inside the animal facility are not worn outside the animal facility.
  - d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
11. 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.
12. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
13. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
14. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
15. 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.<sup>13</sup> Whenever practical, supervisors adopt improved engineering and work practice controls that reduce the 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 animal facility 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 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, or 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 (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.
  - d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
- 16. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
- 17. 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 animal facility.
- 18. 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 animal room 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.
- 19. An effective integrated pest management program is required. See [Appendix G](#).

20. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

**B. Special Practices**

None required.

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

1. Specialized devices or equipment for restraint or containment may be required as determined by appropriate risk assessment.
2. Laboratory coats, gowns, or uniforms are the minimum recommended to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the animal facility.
3. 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 facility waste or decontaminated after use.
4. Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
5. Additional PPE is considered for persons working with large animals.

**D. Animal Facilities (Secondary Barriers)**

1. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - a. External facility doors are self-closing and self-locking.
  - b. Access to the animal facility is restricted.
  - c. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and never propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. The animal facility has a sink for handwashing.
  - a. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
  - b. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
  - c. If open floor drains are provided, the traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, ceilings) are water-resistant.
  - a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
  - b. It is recommended that penetrations in floors, walls, and ceilings be sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
  - c. Internal facility fixtures, such as light fixtures, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - d. External windows are not recommended; if present, they are resistant to breakage. Where possible, windows are sealed. If the animal facility has windows that open, they are fitted with fly screens.
  - e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
4. 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 animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.

5. Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals*.<sup>3</sup>
  - a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
6. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washers have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.

### **Animal Biosafety Level 2**

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

ABSL-2 requires that, in addition to the requirements for ABSL-1, a BSC or other physical containment equipment is used when procedures involve the manipulation of infectious materials or where aerosols or splashes may be created.

Appropriate PPE is worn to reduce exposure to infectious agents, animals, and contaminated equipment. An appropriate occupational health program is in place, as determined by risk assessment.

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

#### **A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are also reviewed and approved by the Institutional Animal Care and Use Committee

(IACUC) and the Institutional Biosafety Committee (IBC), or equivalent resource, as appropriate.

4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); 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.
5. 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](#). Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.
  - a. An animal allergy prevention program is part of the medical surveillance.
  - b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. 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 experimental animals, organisms, biological materials in use, and the work performed.
  - b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the universal biohazard symbol, the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunization, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
10. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - a. Glove selection is based on an appropriate risk assessment.
  - b. Consider the need for bite and/or scratch-resistant gloves.
  - c. Gloves worn inside the animal facility are not worn outside the animal facility.
  - d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
11. 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.
12. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.

13. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
14. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
15. 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, supervisors adopt improved engineering and work practice controls that reduce the 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 animal facility 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, or 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 (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.
  - d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

16. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
17. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Decontaminate all potentially infectious materials before transport or disposal using an effective method. 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 animal facility.
18. 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 animal room 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.
19. An effective integrated pest management program is required. See [Appendix G](#).
20. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

## **B. Special Practices**

1. Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
2. All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a

combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.

- a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.
  - b. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas. Cages are decontaminated prior to washing.
3. Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, and state requirements.
    - a. Equipment is decontaminated before repair, maintenance, or removal from the animal facility. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
    - b. Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, and for major renovations or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
    - c. Decontamination processes are verified on a routine basis.
  4. Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the animal facility supervisor and any other personnel designated by the institution. Appropriate records are maintained.

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

1. Properly maintained BSCs and other physical containment devices or equipment are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include the necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. A risk assessment dictates the type of other physical containment devices used when BSCs may not be suitable.
  - a. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with micro-isolator lids or other equivalent primary containment systems for larger animals.

- b. If used, actively ventilated caging systems are designed to contain microorganisms. Exhaust plenums for these systems are sealed. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positively pressurized if the exhaust fan fails. The system is also alarmed to indicate operational malfunctions. Exhaust HEPA filters and filter housings are certified annually.
2. Protective clothing, such as gowns, uniforms, scrubs, or laboratory coats, and other PPE are worn while in the areas where infectious materials and/or animals are housed or manipulated.
  - a. Scrubs and uniforms are removed before leaving the animal facility.
  - b. Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
  - c. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. 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 from infectious or other hazardous materials when the animal or microorganisms is handled outside the BSC or another containment device. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
4. Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
5. Additional PPE is considered for persons working with large animals.
6. Based on the pathogen and work performed, respiratory protection may be considered for staff enrolled in a properly constituted respiratory protection program.

**D. Animal Facilities (Secondary Barriers)**

1. ABSL-2 facilities should be separated from the general traffic patterns of the building and restricted, as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - a. External facility doors are self-closing and self-locking.
  - b. Access to the animal facility is restricted.
  - c. Doors to areas where infectious materials and/or animals are

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

2. A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink is also available for handwashing at the exit from each segregated area.
  - a. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
  - b. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
  - c. If open floor drains are provided, the traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
  - a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
  - b. Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
  - c. Internal facility fixtures, such as light fixtures, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - d. External windows are not recommended; if present, they are sealed and resistant to breakage.
  - e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
4. Furniture is minimized and 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 animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
5. Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals*.<sup>3</sup>
- a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
  - b. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways.
  - c. A ducted exhaust air ventilation system is provided.
  - d. Exhaust air is discharged to the outside without being recirculated to other rooms.
6. Mechanical cage washers have a final rinse temperature of at least 180°F. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.
7. 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 areas, and other possible airflow disruptions.
  - b. BSCs can be connected to the animal facility 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 animal facility 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](#).
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 on a replacement schedule determined by a risk assessment.

9. An autoclave is present in the animal facility to facilitate decontamination of infectious materials and waste. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.

### **Animal Biosafety Level 3**

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

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

ABSL-3 requires that in addition to the requirements for ABSL-2, all procedures are conducted in BSCs or by use of other physical containment equipment. Inward airflow at the containment boundary is maintained. Handwashing sinks are capable of hands-free operation.

Appropriate PPE is worn to reduce exposure to infectious agents, animals, and contaminated equipment.

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

#### **A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are also reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC), or equivalent resource, as appropriate.

4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); 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.
5. Personal health status may affect an individual's susceptibility to infection, 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](#). Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.
  - a. An animal allergy prevention program is part of the medical surveillance.
  - b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. 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 experimental animals, organisms, biological materials in use, and the work performed.
  - b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the universal biohazard symbol, the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunization, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
10. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - a. Glove selection is based on an appropriate risk assessment.
  - b. Consider the need for bite and/or scratch-resistant gloves.
  - c. Gloves worn inside the animal facility are not worn outside the animal facility.
  - d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated facility waste.
11. 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.
12. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.

13. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
14. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
15. 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, supervisors adopt improved engineering and work practice controls that reduce the 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 animal facility 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, or 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 (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.
  - d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

16. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
17. 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 animal facility.
18. 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 animal room 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.
19. An effective integrated pest management program is required. See [Appendix G](#).
20. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

**B. Special Practices**

1. Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
2. A system is established for reporting and documenting near misses, animal facility accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
3. Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the animal facility director, facility supervisor, institutional management, and

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

4. Only necessary equipment and supplies are recommended to be taken inside the animal facility.
5. All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.
  - a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.
  - b. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas.
6. Biological materials that are to remain in a viable state during removal from the animal facility 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 facility by authorized personnel. Once removed, the primary container is opened within a BSC in BSL-3 or ABSL-3 containment unless a validated inactivated method is used. See [Appendix K](#). The inactivation method is documented in-house with viability testing data to support the method.
7. Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, state, and federal requirements.
  - a. Equipment is decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or manipulated. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
  - b. Decontamination of an entire animal room is considered 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 animal room is based on the risk assessment.
  - c. Decontamination processes are verified on a routine basis.

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

1. Properly maintained BSCs and other physical containment devices or equipment are used for manipulations of infectious materials and animals as determined by risk assessment.
  - a. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with micro-isolator lids, open cages placed in inward flow ventilated enclosures, HEPA filter isolators and caging systems, or other equivalent primary containment systems.
    - i. Actively ventilated caging systems are designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems are sealed to prevent the escape of microorganisms if the ventilation system becomes static, and the exhaust is HEPA-filtered. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system is alarmed to indicate operational malfunctions.
  - b. When animals cannot be housed in ventilated containment cages/units, certain features of the animal room act as the primary barriers. The procedures in place include how workers are protected from agents shed by the animals (e.g., PPE enhancements) as well as how the environment is protected from such agents through the use of biocontainment enhancements such as some combination of boot or PPE change or surface decontamination at the door, a personal shower at the room level, and/or other procedures.
2. Special consideration is given to the potential for cross-contamination when open caging is used. See [Appendix D](#) for additional information.
3. Personnel within the animal facility wear protective clothing, such as uniforms or scrubs.
  - a. Disposable PPE such as non-woven, olefin cover-all suits, or wrap-around or solid-front gowns are worn over this clothing before entering areas where infectious materials and/or animals are housed or manipulated. Front-button, laboratory coats are unsuitable.
  - b. Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.

- c. Disposable PPE is removed when leaving the areas where infectious materials and/or animals are housed or manipulated. Scrubs and uniforms are removed before leaving the animal facility.
  - d. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
4. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate head covering, eye, face, and respiratory protection. To prevent cross-contamination, boots, shoe covers, or other protective footwear are used where indicated and disposed of or decontaminated after use.
  5. Head covering, eye protection, and face protection are disposed of with other contaminated animal facility waste or decontaminated after use.
  6. Procedures may require wearing two pairs of gloves (i.e., double-glove). Change outer gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  7. Additional PPE is considered for persons working with large animals.

**D. Animal Facilities (Secondary Barriers)**

1. ABSL-3 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - a. External facility doors are self-closing and self-locking.
  - b. Access to the animal facility is restricted.
  - c. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and are never propped open.
  - d. Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Exit showers may be considered based on risk assessment. An additional double-door anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.
2. A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a handwashing sink is also available near the exit from each segregated area.

- a. The sink is hands-free or automatically operated.
  - b. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
  - c. Sink traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
  - d. Floor drains are maintained and filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
- a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases. Floors slope to drain, if present.
  - b. Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, outlets, switch plates, and doorframes, to facilitate pest control, proper cleaning, and decontamination. Walls, floors, and ceilings form a sanitizable and sealed surface.
  - c. Internal facility fixtures, such as light fixtures, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - d. External windows are not recommended; if present, they are sealed and resistant to breakage.
  - e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
4. Furniture is minimized and 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 animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.

5. Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.<sup>3</sup>
  - a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
  - b. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A visual monitoring device, which confirms directional airflow, is provided at the animal room entrance.
  - c. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from “clean” areas and toward “contaminated” areas.
  - d. The exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA-filtered.
  - e. The ABSL-3 animal facility is designed such that under failure conditions the airflow will not be reversed at the containment barrier. Alarms are considered to notify personnel of ventilation and HVAC system failure.
6. Cages are decontaminated prior to removal from the containment barrier and prior to washing in a mechanical cage washer. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.
7. 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 areas, and other possible airflow disruptions.
  - b. BSCs can be connected to the animal facility 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 animal facility 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 animal room.
8. Equipment that may produce infectious aerosols is contained in primary barrier devices that exhaust air through HEPA filtration, or other equivalent technology, before being discharged into the animal facility. These HEPA filters are tested annually and replaced as needed.
  9. All vacuum lines are protected with HEPA filters, or their equivalent, or are capped. 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. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.
  10. An autoclave is available within the containment barrier. The autoclave is utilized to decontaminate infectious materials and waste before moving these materials to the other areas of the facility. If not within the containment barrier, special practices are developed for the transport of infectious materials to designated alternate locations for decontamination. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.
  11. The ABSL-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.
  12. Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These 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 animal room isolation; final HEPA filtration of the animal room exhaust air; animal room effluent decontamination; containment of other piped services; or advanced access control devices, such as biometrics.

## **Animal Biosafety Level 4**

Animal Biosafety Level 4 (ABSL-4) is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening 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 ABSL-4 containment are handled at this level until sufficient data are obtained to re-designate the level. Animal care staff receive specific and thorough training in handling extremely hazardous, infectious agents and infected animals. Animal care staff understand the primary and secondary containment functions of standard and special practices, containment equipment, and facility design characteristics. All animal care staff and supervisors are competent in handling animals, agents, and procedures requiring ABSL-4 containment. The animal facility director and/or supervisor control(s) access to the ABSL-4 animal facility in accordance with institutional policies.

There are two models for ABSL-4 facilities:

1. Cabinet Facility: All handling of agents, infected animals, and housing of infected animals is performed in Class III BSCs. See [Appendix A](#); and
2. Suit Facility: Personnel wear a positive-pressure suit. The animal room maintains negative pressure relative to the surrounding areas and have HEPA-filtered supply and exhaust systems. A site-specific risk assessment that considers the agent, the potential for agent shedding, and aerosol generation from infected animals is conducted to determine appropriate animal housing. Most infected animals are housed in a primary containment system and handled under a primary barrier system such as a Class II BSC or another containment system.

ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. However, ABSL-4 cabinet and suit facilities have special engineering and design features to prevent microorganisms from dissemination into the environment and to protect personnel.

The ABSL-4 cabinet facility is distinctly different from an ABSL-3 facility containing a Class III BSC.

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

### **A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.

2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are also reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC), or equivalent resource, as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); 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.
5. 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](#). Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.

- a. An animal allergy prevention program is part of the medical surveillance.
  - b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. 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 experimental animals, 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, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the universal biohazard symbol, the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, general occupational health requirements (e.g., immunization, respiratory protection), PPE requirements and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
- a. Glove selection is based on an appropriate risk assessment.
  - b. Inner gloves worn inside the animal facility are not worn outside the animal facility.
  - 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 animal facility waste.

10. 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.
11. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
12. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
13. 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, supervisors adopt improved engineering and work practice controls that reduce the 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 animal facility 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, or 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 (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.

- d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
- 14. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
- 15. 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 animal facility.
- 16. All wastes from the animal room, including animal tissues, carcasses, and bedding are transported from the animal room in leak-proof, covered containers for appropriate disposal consistent with applicable institutional, local, and state requirements. See B. Special Practices, #7 in the following sub-section for additional details.
- 17. An effective integrated pest management program is required. See [Appendix G](#).
- 18. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

**B. Special Practices**

- 1. All persons entering the animal facility 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 animal 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 the animal facility.
- 2. All persons who enter operational animal 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 facility.
  - 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. The facility supervisor is responsible for ensuring that, prior to working independently in ABSL-4 containment, personnel demonstrate high

proficiency in standard and special microbiological practices, and techniques for working with agents requiring ABSL-4 containment.

4. A system is established for reporting and documenting near misses, accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
5. Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policy. All incidents are reported to the animal facility director, facility supervisor, institutional management, and appropriate facility safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.
6. Biological materials that are to remain in a viable state during removal from the animal facility 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 facility by authorized personnel. These materials are transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, the primary container is not opened outside BSL-4 or ABSL-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 wastes (including animal tissues, carcasses, and contaminated bedding) and other materials are decontaminated by a verified method before removal from the ABSL-4 facility.
8. Equipment is routinely decontaminated and is decontaminated before repair, maintenance, or removal from the animal facility. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas. Cages are autoclaved or thoroughly decontaminated before they are cleaned and washed.
  - a. Equipment (e.g., sensitive electronic, medical, or routine husbandry equipment) or material that might be damaged by high temperatures or steam is decontaminated using an effective and verified procedure such as a gaseous or vapor method in a sealable airlock or chamber designed for this purpose.
9. Procedures to reduce possible worker exposure are instituted, such as use of squeeze cages, working only with anesthetized animals, or other appropriate practices. Personnel assigned to work with infected animals may be required to work in pairs as directed by institutional policies.

10. A logbook, or other means of documenting the date and time of all persons entering and leaving the animal facility, is maintained.
11. While the facility is operational, personnel enter and exit the animal facility 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 facility use animal facility clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves, as appropriate. All persons leaving the animal facility are required to take a personal body shower. Used animal facility clothing and other waste, including gloves, are treated as contaminated materials and decontaminated before laundering or disposal.
12. After the facility has been completely decontaminated by verification of a validated method, necessary staff may enter and exit the animal facility 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 animal rooms and animal facilities are operating according to established parameters.
14. Only necessary equipment and supplies are stored inside the animal facility. All equipment and supplies taken inside the facility are decontaminated before removal from the laboratory.
  - a. Supplies and materials that are not brought into the animal facility 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 Facility*

1. All procedures involving the manipulation of infectious animals and 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 animal room 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 for annual filter recertification.
  - 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 the cabinet 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. Class III cabinet gloves are inspected for leaks periodically 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 an ABSL-4 suit facility, materials may be placed into and removed from the Class III BSC via the suit facility.
8. Restraint devices and practices that reduce the risk of exposure during animal manipulations are used where practicable (e.g., physical restraint devices, chemical restraint medications, mesh, or Kevlar gloves).
9. Workers in the animal facility wear protective animal facility clothing with a solid front, such as tie-back or wrap-around gowns, scrubs, or coveralls. Additional PPE may be required based on 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.
10. Disposable gloves are worn underneath cabinet gloves to protect the worker from exposure should a break or tear occur in a cabinet glove.

#### *Suit Facility*

1. All procedures involving the manipulation of infectious materials or infected animals are conducted within a BSC or other physical containment devices.
2. Infected animals are housed in a primary containment system. Primary containment systems include: actively ventilated caging systems; open cages placed in ventilated enclosures; solid wall and bottom cages covered with micro-isolator lids and opened in laminar flow hoods or HEPA-filtered downdraft tables; or other equivalent primary containment systems.
  - a. Actively ventilated caging systems are designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems are sealed to prevent the escape of microorganisms if the ventilation system becomes static, and the exhaust is HEPA-filtered. These HEPA filters are tested annually and replaced as needed. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system is alarmed to indicate operational malfunctions.

3. Infected animals may be housed in open cages within a dedicated animal-holding room that serves as the primary barrier. A room serving as a primary barrier is air-tight and capable of being decontaminated using fumigation. If animals are to be contained in a dedicated animal-holding room serving as the primary barrier, the following conditions are met:
  - a. Prior to fumigation of the animal-holding room, cages may be removed for autoclaving or chemical decontamination.
  - b. Caging is chosen to reduce the amount of animal detritus that can be thrown out of the cage and onto the floor of the animal holding room.
  - c. The flow of personnel, material, and equipment is directed in order to minimize the spread of contamination from the animal-holding room into adjacent areas of the animal facility.
4. When large animals cannot be housed in a primary containment system or ventilated containment cages/units, certain features of the animal room (e.g., HEPA exhaust filters and the sealed and pressure-tested room surfaces) act as the primary barriers.
  - a. Loose-housed or open penned animals may require ABSL-3Ag or ABSL-4Ag containment. See [Appendix D](#) for additional information.
5. Equipment that may produce aerosols is used within primary containment devices that exhaust air through HEPA filtration before being discharged into the animal room or facility exhaust system. These HEPA filters are tested annually and replaced as needed.
6. All procedures are conducted by personnel wearing a one-piece, positive-pressure supplied-air suit.
  - a. All persons don animal facility 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 operations to remove gross contamination and minimize further contamination of the animal room.
  - 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 animal facility 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. Animal Facilities (Secondary Barriers)**

##### *Cabinet Facility*

1. The ABSL-4 cabinet facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - a. Facility access is restricted. Facility doors are lockable.
  - b. Exit from the animal facility 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 facility.
2. An automatically activated emergency power source is provided at a minimum for the animal facility 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).
  - b. The emergency power system(s) is tested at least annually.
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 from the cabinet room to the inner change rooms. A sink is provided in the outer change room.
5. An eyewash station is readily available in the animal area.
6. Walls, floors, and ceilings of the cabinet facility 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 facility are sealed.
  - b. Openings around doors into the facility are minimized and capable of being sealed to facilitate decontamination.

7. Services and plumbing that penetrate the facility walls, floors, or ceiling are installed to ensure that no backflow from the facility 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 animal areas are covered with a non-porous material that can be easily cleaned and decontaminated as appropriate and sealed to prevent harboring of insects/vermin.
  - d. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
9. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
10. Windows are break-resistant and sealed.
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, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
  - b. BSCs can be connected to the animal facility 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 animal facility 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 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 room system.
  - a. The supply and exhaust components of the ventilation system are designed to maintain the cabinet facility at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the facility.
  - b. Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans are interlocked to prevent positive pressurization of the facility.
  - 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 facility may be verified prior to entry and during regular checklist procedures. Visual monitoring is also in place within the cabinet room.
  - d. Supply air to and exhaust air from the cabinet room, 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 room 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 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 room(s). Access to the exit side of the pass-through is limited

to those with authorized access to the animal facility and with specific clearance, if required.

15. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet facility 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 annually or more often as 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 facility. Autoclaves that open outside of the facility 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 facility 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.
  - b. The size of the autoclave is sufficient to accommodate the expected volume of waste, size of equipment and cages, and any future programmatic needs.
17. Cages are decontaminated prior to removal from the cabinet. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.
18. The animal 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.

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

#### *Suit Facility*

1. The ABSL-4 suit facility may be located in a separate building or a clearly demarcated and isolated zone within a building. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
  - a. Facility access is restricted. Facility doors are lockable.
  - b. Entry into the animal facility is through an airlock fitted with airtight doors.
  - c. Exit from the facility 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 facility to allow the personnel to safely exit the facility.
3. A chemical shower is provided to decontaminate the surface of the positive-pressure suit before the worker leaves the facility. 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 animal facility 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 facility.
6. Hands-free sinks inside the animal facility are placed near procedure areas.

7. An eyewash station for use during maintenance is readily available in the animal area.
8. Walls, floors, and ceilings of the animal facility 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 animal room(s), suit storage room, and the inner change room are sealed.
9. Services and plumbing that penetrate the facility walls, floors, or ceiling are installed to ensure that no backflow from the facility 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, are sealed up to the second filter, and have protection against insect and animal intrusion.
10. Decontamination of the entire facility is performed using a validated gaseous or vapor method when there has been a significant change in facility 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 facility.
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 animal areas are covered with a non-porous material that can be easily cleaned and decontaminated as appropriate and sealed to prevent harboring of insects/vermin.
  - d. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
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 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 areas, and other possible airflow disruptions.
  - b. BSCs can be connected to the animal facility 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. Class IIA or IIC BSC exhaust can be safely recirculated back into the facility 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 animal room.
15. Central vacuum systems are discouraged. If there is a central vacuum system, it does not serve areas outside the ABSL-4 facility. 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 animal room.
  - a. The supply and exhaust components of the ventilation system are designed to maintain the ABSL-4 facility at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the facility.
  - b. Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans are interlocked to prevent positive pressurization of the facility.
  - 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 facility may be verified prior to entry and during

regular checklist procedures. Visual monitoring is also in place within containment.

- d. Supply air to the animal facility, including the decontamination shower, passes through a HEPA filter. All exhaust air from the suit facility, 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 areas where infectious materials and/or animals are housed or manipulated 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 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.
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 animal facility. Access to the exit side of the pass-through is limited to those individuals authorized to be in the animal facility and provided with appropriate clearance if required.
  18. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the facility 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 as 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 facility. Autoclaves that open outside of the facility 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 facility 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 the 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.
  - b. The size of the autoclave is sufficient to accommodate the expected volume of waste, size of equipment and cages, and any future programmatic needs.
20. Cages are decontaminated prior to removal from the animal facility. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.
  21. The ABSL-4 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.
  22. Appropriate communication systems are provided between the facility and the outside (e.g., voice, fax, video, and computer). Provisions for emergency communication and emergency access or egress are developed and implemented.
  23. Facilities housing animals in open caging have the following design elements:
    - a. Access to the animal holding room from service corridors outside of the containment space requires passage through two sets of doors, and the innermost door is an air pressure resistant (APR) door.
    - b. For any animal holding room considered to be a primary barrier, the APR door(s) providing direct ingress from the exterior service corridor is fitted with appropriate and redundant lockout mechanisms to prevent access when the animal-holding room is contaminated and in use. There is more than one mechanism to ensure that this primary barrier door cannot be opened when the animal room is contaminated and the APR door does not serve as an emergency exit from the animal facility. The APR door is appropriately tested to

demonstrate that in the closed, locked-out mode, the door provides an air-tight barrier proven by pressure decay testing or other equivalent method(s).

- c. Any door(s) allowing access into an internal corridor from which there is direct ingress to an animal holding room is fitted with either: 1) an APR door; or 2) a non-APR door, providing directional airflow is maintained from the corridor space into the animal room. For the purpose of fumigation, animal rooms equipped with non-APR doors opening into the adjacent interior corridors are considered one space (i.e., areas between air-tight doors are fumigated together).
  - d. Any door(s) used for access to the out-of-containment service corridor (the secondary barrier) are self-closing and of solid construction, designed not to corrode, split, or warp.
  - e. Access to the service corridor inside the secondary barrier is restricted and strictly controlled when animal rooms are in use. Whenever possible, the secondary barrier door(s) is fitted with safety interlock switches designed to prevent it from opening when an animal-holding room door (the primary barrier) is opened following room decontamination; if interlock devices cannot be used, specific administrative procedures are implemented to control access to the service corridor.
  - f. The out-of-containment service corridor maintains a negative pressure (inward directional airflow) relative to adjoining traffic corridors.
24. Loose-housed or open penned animals may be subject to the requirements of ABSL-3Ag or ABSL-4Ag. See [Appendix D](#) for additional information.

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