Chapter 5

Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for laboratory animal. Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents in vivo and in vitro are comparable.

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol driven risk assessment.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., Guide for the Care and Use of Laboratory Animals\(^1\) and Laboratory Animal Welfare Regulations\(^2\)) and that appropriate species have been selected for animal experiments. In addition, the organization must have an occupational health and safety program that addresses potential hazards associated with the conduct of laboratory animal research. The following publication by the Institute for Laboratory Animal Research (ILAR), Occupational Health and Safety in the Care of Research Animals\(^3\) is most helpful in this regard. Additional safety guidance on working with non-human primates is available in the ILAR publication, Occupational Health and Safety in the Care and Use of Nonhuman Primates.\(^4\)

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

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

In addition to the animal biosafety levels described in this chapter the USDA has developed facility parameters and work practices for handling agents of agriculture significance. Chapter 12 includes a discussion on Animal Biosafety Level-3 Agriculture (ABSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. Chapter 12 also describes some of the enhancements beyond BSL/ABSL-3 that may be required by USDA-APHIS when working in the laboratory or vivarium with certain veterinary agents of concern.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in this chapter. The reader is referred to Chapter 13 for more information on the Arthropod Containment Guidelines.

**Animal Biosafety Level 1**

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

ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment (See Chapter 2).

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

The following standard practices, safety equipment, and facility requirements apply to ABSL-1:

**A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations.

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC)\(^5\) and the Institutional Biosafety Committee.
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security-sensitive agent information should be posted in accordance with the institutional policy.
Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.  

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.

Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

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

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

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

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.

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

14. An effective integrated pest management program is required. See Chapter 15.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate all potentially infectious materials before disposal using an effective method.

**B. Special Practices**

None required.

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

1. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.
2. Special containment devices or equipment may be required as determined by appropriate risk assessment.

Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing.

Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.

3. Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, face shields, etc.) as needed.

4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

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

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.
Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. The animal facility must have a sink for hand washing.

Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.

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

Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals.¹ No recirculation of exhaust air should occur. It is recommended that animal rooms have inward directional airflow.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

**Animal Biosafety Level 2**

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

ABSL-2 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

**A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations.

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.
Prior to beginning a study animal protocols must also be reviewed and approved by the IACUC and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

   The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.

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

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

   Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

   Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

   Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and
Exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.¹ ³ ⁴

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.

Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

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

d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

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

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.

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

14. An effective integrated pest management program is required See Chapter 15.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate of all potentially infectious materials before disposal using an effective method.

**B. Special Practices**
1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

When appropriate, a baseline serum sample should be stored.

2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

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

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

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

Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must contain a universal biohazard label.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

4. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas.

Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
5. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

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

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols or splashes. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents, or larger cages placed in inward flow ventilated enclosures or other equivalent primary containment systems for larger animal cages.

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

Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.

Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for anticipated splashes/ sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

Persons having contact with the NHP should assess risk of mucous membrane exposure and wear appropriate protective equipment (e.g., masks, goggles, face shields, etc.) as needed. Respiratory protection is worn based upon risk assessment.
4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials.

Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

Access to the animal facility is restricted.

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

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility.

If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.

Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.
Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning.

Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, windows should be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals.\textsuperscript{1} The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage/equipment cleaning process.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. Correct performance of the BSCs should be recertified at least once a year.

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

12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.

13. An autoclave should be considered in the animal facility to facilitate decontamination of infectious materials and waste.

14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

**Animal Biosafety Level 3**

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

ABSL-3 laboratory has special engineering and design features.

ABSL-3 requires that 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment.
Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3

A. Standard Microbiological Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations.

Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.

Prior to beginning a study animal protocols must also be reviewed and approved by the IACUC and the Institutional Biosafety Committee.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures.

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

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.

Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women
of child-bearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.1,3,4

6. Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated.

All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

Gloves are worn to prevent skin contact with contaminated, infectious/ and hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.

Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

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

d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

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

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.

14. An effective integrated pest management program is required. See Chapter 15.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.

Decontaminate of all potentially infectious materials before disposal using an effective method.

**B. Special Practices**

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

2. All procedures involving the manipulation of infectious materials, handling infected animals or the generations of aerosols must be conducted within BSCs or other physical containment devices when practical.

When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

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

3. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems (such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems).

4. Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate when operational malfunctions occur.
5. A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials and/or animals are housed or are manipulated (e.g. autoclave, chemical disinfection, or other approved decontamination methods).

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

Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) before removal from the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method.

It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or are manipulated, preferably within the caging system.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

6. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas.

Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

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

1. Properly maintained BSCs, and other physical containment devices or equipment, should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.
The risk of infectious aerosols from infected animals or bedding can be reduced through the use of primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets; ventilated cage rack systems; or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.

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

Protective clothing such as uniforms or scrub suits is worn by personnel within the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.

Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility.

Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Appropriate eye, face and respiratory protection are worn by all personnel entering areas where infectious materials and/or animals are housed or are manipulated. To prevent cross contamination boots, shoe covers, or other protective footwear, are used where indicated.

Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

4. Gloves are worn to protect hands from exposure to hazardous materials.

A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

Procedures may require the use of wearing two pairs of gloves (double-glove). Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary.

Gloves must not be worn outside the animal rooms.

Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials.
Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.

   Access to the animal facility is restricted.

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

   Entry into the containment area is via a double-door entry which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-door autoclave may be provided for movement of supplies and wastes into and out of the facility.

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated.

   If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area.

   Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.
Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface.

Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases.

Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation to the facility should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*. The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.

This system creates directional airflow which draws air into the animal room from "clean" areas and toward "contaminated" areas.

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered.

Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under
failure conditions the airflow will not be reversed. Audible alarms should be considered to notify personnel of ventilation and HVAC system failure.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage cleaning process.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with its proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance.

Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.

All BSCs should be used according to manufacturers’ recommendations. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

12. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials designated alternate location/s within the facility.
13. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

15. The ABSL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.

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

Animal Biosafety Level 4

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring ABSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or to re-designate the level. Animal care staff must have specific and thorough training in handling extremely hazardous, infectious agents and infected animals. Animal care staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All animal care staff and supervisors must be competent in handling animals, agents and procedures requiring ABSL-4 containment. Access to the animal facility within the ABSL-4 laboratory is controlled by the animal facility director and/or laboratory supervisor in accordance with institutional policies.

There are two models for ABSL-4 laboratories:

(1) A Cabinet Laboratory where all handling of agents, infected animals and housing of infected animals must be performed in Class III BSCs (See Chapter 9).

(2) A Suit Laboratory where personnel must wear a positive pressure protective suit (See Chapter 9); infected animals must be housed in ventilated enclosures with inward directional airflow and HEPA filtered exhaust; infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.
ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. However ABSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment and personnel. The ABSL-4 cabinet laboratory is distinctly different from an ABSL-3 laboratory containing a Class III BSC. The following standard and special safety practices, equipment, and facilities apply to ABSL-4.

A. **Standard Microbiological Practices**

1. The animal facility directors must establish and enforce policies, procedures, and protocols for biosafety, biosecurity and emergency situations within the ABSL-4 laboratory.

   The animal facility director and/or designated institutional officials are responsible for enforcing the policies that control access to the ABSL-4 facility. Laboratory personnel and support staff must be provided appropriate occupational medical service including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory acquired infections. Facility supervisors should ensure that medical staffs are informed of potential occupational hazards within the animal facility including those associated with research, animal husbandry duties, animal care and manipulations.

   An ABSL-4 laboratory specific, biosafety manual must be prepared in consultation with the animal facility director, the laboratory supervisor, and the biosafety advisor. The biosafety manual must be available and accessible. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.

   Prior to beginning a study, appropriate policies and procedures for animal welfare during the conduct of research, must be developed and approved by the IACUC. The biosafety official, the IBC and/or other applicable committees, are responsible for review of protocols and polices to prevent hazardous exposures to personnel who manipulate and care for animals.

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

   All persons leaving the BSL-4/ABSL-4 laboratory are required to take a personal body shower.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Needles and syringes or other sharp instruments are limited for use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal and placed as close to the work site as possible.

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

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

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

6. Perform all procedures to minimize the creation of splashes and/or aerosols. Procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets, or other physical containment devices. When procedures cannot be performed in a BSC, alternate containment equipment should be used.

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

Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the animal facility director, laboratory supervisor, institutional
management and appropriate facility safety personnel. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

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

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

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

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

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory and the animal room/s when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.¹,³,⁴

10. An effective integrated pest management program is required. See Chapter 15.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure
evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

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

B. Special Practices

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

Only persons whose presence in the laboratory or individual animal rooms is required for scientific or support purposes should be authorized to enter.

Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the ABSL-4 laboratory must be maintained.

While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. Laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves, must be used by all personnel entering the laboratory.

All persons leaving the ABSL-4 laboratory are required to take a personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering or disposal.

After the laboratory has been completely decontaminated by validated method, necessary staff may enter and exit the laboratory without following the clothing change and shower requirements described above. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.
2. Animal facility personnel and support staff must be provided occupational medical services, including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-acquired illnesses. An essential adjunct to an occupational medical system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired illnesses.

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

4. The animal facility supervisor is responsible for ensuring that animal personnel:
   
a. Receive appropriate training in the practices and operations specific to the animal facility, such as animal husbandry procedures, potential hazards present, manipulations of infectious agents, necessary precautions to prevent potential exposures.
   
b. Demonstrate high proficiency in standard and special microbiological practices, and techniques before entering the ABSL-4 facility or working with agents requiring ABSL-4 containment.
   
c. Receive annual updates and additional training when procedure or policy changes occur. Records are maintained for all hazard evaluations and employee training.

5. Removal of biological materials that are to remain in a viable or intact state from the ABSL-4 laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside ABSL-4 containment unless inactivated by a validated method.

6. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Cages are autoclaved or thoroughly decontaminated before they are cleaned and washed.
   
a. All equipment and contaminated materials must be decontaminated before removal from the animal facility. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the animal facility.
   
b. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as gaseous or vapor method in an airlock or chamber designed for this purpose.
c. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory. Spills and accidents of potentially infectious materials must be immediately reported to the animal facility and laboratory supervisors or personnel designated by the institution.

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

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

9. Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the ABSL-4 laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel according to institutional policies.

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

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

Cabinet Laboratory

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

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

The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.
The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

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

Class III cabinet gloves must be inspected for leaks periodically and changed if necessary. Gloves should be replaced annually during cabinet recertification.

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

Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups.

The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.

The Class III cabinet must be certified at least annually.

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

2. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls must be worn by workers when in the laboratory. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. All protective clothing must be removed in the dirty side change room before showering. Reusable laboratory clothing must be autoclaved before being laundered.

3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eye glasses must be decontaminated before removal thought the personal body shower.
4. Gloves must be worn to protect against breaks or tears in the cabinet gloves. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

**Suit Laboratory**

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

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

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

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

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations.

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

3. Inner gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to personal shower. Dispose of used gloves with other contaminated waste.

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

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

**Cabinet Laboratory**
1. The ABSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the ABSL-4 facility must be arranged to ensure sequential passage through an inner (dirty) change area, personal shower and outer (clean) change room prior to exiting the room(s) containing the Class III BSC(s).

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on an uninterrupted power supply (UPS).

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

2. A hands-free sink must be provided near the doors of the cabinet room(s) and the inner change rooms. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC and the inner (dirty) change room must be connected to the wastewater decontamination system.

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

All penetrations in the internal shell of the laboratory and inner change room must be sealed.

Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

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

Services that penetrate the walls, floors or ceiling, plumbing or otherwise, must ensure that no backflow from the laboratory occurs. Services must be sealed and be provided with redundant backflow prevention. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and are sealed up to the second filter.
Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment. Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment of the biological agents in use.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated.

5. Windows must be break-resistant and sealed.

6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory.

9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if each individual laboratory system is isolated by gas tight dampers and HEPA filters. The supply and exhaust components of the ventilation system must be designed to maintain the ABSL-4 laboratory at negative pressure to surrounding areas and provide differential pressure/directional airflow between adjacent areas within the laboratory.

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

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified.

Supply air to and exhaust air from the cabinet room, inner change room, and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes.
All HEPA filters should be located as near as practicable to the cabinet laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings should be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer’s recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass though shall be limited to those individuals authorized to be in the ABSL-4 laboratory.

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

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often as required by institutional policy.

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

13. A double-door autoclave must be provided for decontaminating waste or other materials passing out of the cabinet room. Autoclaves that open outside of the laboratory must be sealed to the wall. This bioseal must be durable, airtight, and sealed to the wall. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door can only be opened after the autoclave decontamination cycle has been completed.
Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The ABSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.

15. Appropriate communication systems must be provided between the ABSL-4 laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress must be considered.

**Suit Laboratory**

1. The ABSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

   Rooms in the facility must be arranged to ensure exit sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

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

   A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the ABSL-4 laboratory. In the event of an emergency exit or failure of chemical shower, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

   An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on a UPS.

   A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. Sinks inside the ABSL-4 laboratory must be placed near procedure areas and contain traps and be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the ABSL-4 laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed.

Drains, if present in the laboratory floor, must be connected directly to the liquid waste decontamination system. Sewer vents and other service lines must be protected by two HEPA filters in series and have protection against insect and animal intrusion.

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

Decontamination of the entire laboratory must be performed using a validated gaseous or vapor method when there have been significant changes in laboratory usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning, decontamination and unencumbered movement of personnel. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated. Sharp edges and corners should be avoided.

5. Windows must be break-resistant and sealed.

6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Central vacuum systems are not recommended.

If, however, there is a central vacuum system, it must not serve areas outside the ABSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.
8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.

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

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

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

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified.

Supply air to the ABSL-4 laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the BSL-4/ABSL-4 suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.

All HEPA filters must be located as near as practicable to the areas where infectious materials and/or animals are housed or are manipulated in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings are designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports; and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer’s recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave
can be safely removed from the ABSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the ABSL-4 laboratory.

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

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often as required by institutional policy.

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

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

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

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The ABSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified. Verification criteria should be modified as necessary by operational experience.

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

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


5. National Institutes of Health, Office of Laboratory Animal Welfare. Public Health Service policy on humane care and use of laboratory animals, Bethesda (MD); The National Institutes of Health (US); 2000.
<table>
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<tr>
<th>ABSL</th>
<th>AGENTS</th>
<th>PRACTICES</th>
<th>PRIMARY BARRIERS AND SAFETY EQUIPMENT</th>
<th>FACILITIES (SECONDARY BARRIERS)</th>
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</table>
| 1    | Not known to consistently cause diseases in healthy adults | Standard animal care and management practices, including appropriate medical surveillance programs | As required for normal care of each species | Standard animal facility:  
- No recirculation of exhaust air  
- Directional air flow recommended  
- Hand washing sing is available |
| 2    | • Associated with human disease  
- Hazard: percutaneous exposure, ingestion, mucous membrane exposure | ABSL-1 practice plus:  
- Limited access  
- Biohazard warning signs  
- “Sharps” precautions  
- Biosafety manual  
- Decontamination of all infectious wastes and of animal cages prior to washing | ABSL-1 equipment plus primary barriers:  
- Containment equipment appropriate for animal species  
- PPEs*:  
  - Laboratory coats, gloves, face and respiratory protection as needed | ABSL-1 plus:  
- Physical separation from access corridors  
- Self-closing, double door access  
- Sealed penetrations  
- Sealed windows  
- Autoclave available in facility |
| 3    | • Indigenous or exotic agents with potential for aerosol transmission  
- Disease may have serious health effects | ABSL-2 practice plus:  
- Controlled access  
- Decontamination of clothing before laundering  
- Cages decontaminated before bedding removed  
- Disinfectant foot bath as needed | ABSL-2 equipment plus:  
- Containment equipment for housing animals and cage dumping activities  
- Class I, II, or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols  
- PPEs*:  
  - Appropriate respiratory protection | BSL-2 plus:  
- Physical separation from access corridors  
- Self-closing, double-door access  
- Sealed penetrations  
- Sealed windows  
- Autoclave available in facility |
| 4    | | | UNC does not have ABSL-4 facilities | |

* PPE – Personal Protective Equipment