



## **VERTEBRATE ANIMAL BIOSAFETY LEVEL 3 CRITERIA**

### ***Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition Section V***

#### **Animal Biosafety Level 3**

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

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

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

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

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

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

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are also reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC), or equivalent resource, as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.

5. Personal health status may affect an individual's susceptibility to infection, ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. See Section VII. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.
  - a. An animal allergy prevention program is part of the medical surveillance.
  - b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
  - a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, biological materials in use, and the work performed.
  - b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the universal biohazard symbol, the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunization, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
10. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
  - a. Glove selection is based on an appropriate risk assessment.
  - b. Consider the need for bite and/or scratch-resistant gloves.
  - c. Gloves worn inside the animal facility are not worn outside the animal facility.
  - d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

- e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated facility waste.
11. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
  12. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
  13. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
  14. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
  15. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, supervisors adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions are always taken with sharp items. These include:
    - a. Plasticware is substituted for glassware whenever possible.
    - b. Use of needles and syringes or other sharp instruments is limited in the animal facility and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
      - i. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
      - ii. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
      - iii. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, or the use of forceps to hold the cap when recapping a needle).
      - iv. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
    - c. Non-disposable sharps (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.
    - d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
  16. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
  17. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are

contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.

18. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
  - a. Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
  - b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
19. An effective integrated pest management program is required. See Appendix G.
20. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

## **B. Special Practices**

1. Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
2. A system is established for reporting and documenting near misses, animal facility accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
3. Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the animal facility director, facility supervisor, institutional management, and appropriate facility safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.
4. Only necessary equipment and supplies are recommended to be taken inside the animal facility.
5. All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.
  - a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.
  - b. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas.
6. Biological materials that are to remain in a viable state during removal from the animal facility are placed in a durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the facility by authorized

personnel. Once removed, the primary container is opened within a BSC in BSL-3 or ABSL-3 containment unless a validated inactivated method is used. See Appendix K. The inactivation method is documented in-house with viability testing data to support the method.

7. Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, state, and federal requirements.
  - a. Equipment is decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or manipulated. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
  - b. Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
  - c. Decontamination processes are verified on a routine basis.

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

1. Properly maintained BSCs and other physical containment devices or equipment are used for manipulations of infectious materials and animals as determined by risk assessment.
  - a. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with micro-isolator lids, open cages placed in inward flow ventilated enclosures, HEPA filter isolators and caging systems, or other equivalent primary containment systems.
    - i. Actively ventilated caging systems are designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems are sealed to prevent the escape of microorganisms if the ventilation system becomes static, and the exhaust is HEPA-filtered. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system is alarmed to indicate operational malfunctions.
  - b. When animals cannot be housed in ventilated containment cages/units, certain features of the animal room act as the primary barriers. The procedures in place include how workers are protected from agents shed by the animals (e.g., PPE enhancements) as well as how the environment is protected from such agents through the use of biocontainment enhancements such as some combination of boot or PPE change or surface decontamination at the door, a personal shower at the room level, and/or other procedures.
2. Special consideration is given to the potential for cross-contamination when open caging is used. See Appendix D for additional information.
3. Personnel within the animal facility wear protective clothing, such as uniforms or scrubs.
  - a. Disposable PPE such as non-woven, olefin cover-all suits, or wrap-around or solid-front gowns are worn over this clothing before entering areas where infectious materials and/or animals are housed or manipulated. Front-button, laboratory coats are unsuitable.

- b. Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
  - c. Disposable PPE is removed when leaving the areas where infectious materials and/or animals are housed or manipulated. Scrubs and uniforms are removed before leaving the animal facility.
  - d. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
4. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate head covering, eye, face, and respiratory protection. To prevent cross-contamination, boots, shoe covers, or other protective footwear are used where indicated and disposed of or decontaminated after use.
  5. Head covering, eye protection, and face protection are disposed of with other contaminated animal facility waste or decontaminated after use.
  6. Procedures may require wearing two pairs of gloves (i.e., double-glove). Change outer gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  7. Additional PPE is considered for persons working with large animals.

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

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

- d. Floor drains are maintained and filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
  - a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases. Floors slope to drain, if present.
  - b. Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, outlets, switch plates, and doorframes, to facilitate pest control, proper cleaning, and decontamination. Walls, floors, and ceilings form a sanitizable and sealed surface.
  - c. Internal facility fixtures, such as light fixtures, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
  - d. External windows are not recommended; if present, they are sealed and resistant to breakage.
  - e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
4. Furniture is minimized and can support anticipated loads and uses.
  - a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
  - c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
5. Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.
  - a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
  - b. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A visual monitoring device, which confirms directional airflow, is provided at the animal room entrance.
  - c. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from "clean" areas and toward "contaminated" areas.
  - d. The exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA-filtered.

- e. The ABSL-3 animal facility is designed such that under failure conditions the airflow will not be reversed at the containment barrier. Alarms are considered to notify personnel of ventilation and HVAC system failure.
6. Cages are decontaminated prior to removal from the containment barrier and prior to washing in a mechanical cage washer. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.
7. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
  - a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
  - b. BSCs can be connected to the animal facility exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the animal facility environment if no volatile toxic chemicals are used in the cabinet.
  - c. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.
  - d. Class III BSCs are provided supply air in such a manner that prevents positive pressurization of the cabinet or the animal room.
8. Equipment that may produce infectious aerosols is contained in primary barrier devices that exhaust air through HEPA filtration, or other equivalent technology, before being discharged into the animal facility. These HEPA filters are tested annually and replaced as needed.
9. All vacuum lines are protected with HEPA filters, or their equivalent, or are capped. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. See Appendix A, Figure 11. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.
10. An autoclave is available within the containment barrier. The autoclave is utilized to decontaminate infectious materials and waste before moving these materials to the other areas of the facility. If not within the containment barrier, special practices are developed for the transport of infectious materials to designated alternate locations for decontamination. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.
11. The ABSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified as necessary by operational experience.
12. Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These enhancements may include one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas-tight dampers to facilitate animal room isolation; final HEPA

filtration of the animal room exhaust air; animal room effluent decontamination; containment of other piped services; or advanced access control devices, such as biometrics.