



VERTEBRATE ANIMAL BIOSAFETY LEVEL 2 CRITERIA

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

Animal Biosafety Level 2

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

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

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

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

A. Standard Microbiological Practices

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

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

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

materials before transport or disposal using an effective method. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.

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

B. Special Practices

1. Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
2. All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.
 - a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.
 - b. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas. Cages are decontaminated prior to washing.
3. Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, and state requirements.
 - a. Equipment is decontaminated before repair, maintenance, or removal from the animal facility. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
 - b. Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, and for major renovations or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
 - c. Decontamination processes are verified on a routine basis.

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

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

1. Properly maintained BSCs and other physical containment devices or equipment are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include the necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. A risk assessment dictates the type of other physical containment devices used when BSCs may not be suitable.
 - a. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with micro-isolator lids or other equivalent primary containment systems for larger animals.
 - b. If used, actively ventilated caging systems are designed to contain microorganisms. Exhaust plenums for these systems are sealed. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positively pressurized if the exhaust fan fails. The system is also alarmed to indicate operational malfunctions. Exhaust HEPA filters and filter housings are certified annually.
2. Protective clothing, such as gowns, uniforms, scrubs, or laboratory coats, and other PPE are worn while in the areas where infectious materials and/or animals are housed or manipulated.
 - a. Scrubs and uniforms are removed before leaving the animal facility.
 - b. Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
 - c. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials when the animal or microorganisms is handled outside the BSC or another containment device. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
4. Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
5. Additional PPE is considered for persons working with large animals.
6. Based on the pathogen and work performed, respiratory protection may be considered for staff enrolled in a properly constituted respiratory protection program.

D. Laboratory Facilities (Secondary Barriers)

1. ABSL-2 facilities should be separated from the general traffic patterns of the building and restricted, as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
 - a. External facility doors are self-closing and self-locking.

- b. Access to the animal facility is restricted.
 - c. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and are never to be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink is also available for handwashing at the exit from each segregated area.
 - a. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
 - b. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
 - c. If open floor drains are provided, the traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
 - a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
 - b. Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
 - c. Internal facility fixtures, such as light fixtures, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
 - d. External windows are not recommended; if present, they are sealed and resistant to breakage.
 - e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
4. Furniture is minimized and can support anticipated loads and uses.
 - a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
 - c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
5. Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals*.

- a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
 - b. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways.
 - c. A ducted exhaust air ventilation system is provided.
 - d. Exhaust air is discharged to the outside without being recirculated to other rooms.
6. Mechanical cage washers have a final rinse temperature of at least 180°F. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during the cage/equipment cleaning process.
 7. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
 - a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
 - b. BSCs can be connected to the animal facility exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the animal facility environment if no volatile toxic chemicals are used in the cabinet.
 - c. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.
 8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. See Appendix A, Figure 11. Filters are replaced, as needed, or on a replacement schedule determined by a risk assessment.
 9. An autoclave is present in the animal facility to facilitate decontamination of infectious materials and waste. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.