UNIVERSITY OF South Carolina

LABORATORY BIOSAFETY LEVEL 3 CRITERIA

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

Biosafety Level 3

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

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

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

A. Standard Microbiological Practices

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

5. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
6. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
7. Gloves are worn to protect hands from exposure to hazardous materials.
    a. Glove selection is based on an appropriate risk assessment.
    b. Gloves are not worn outside the laboratory.
    c. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    d. Do not wash or reuse disposable gloves and dispose of used gloves with other contaminated laboratory waste.
8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
9. Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.
10. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
11. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
12. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
    a. Plasticware is substituted for glassware whenever possible.
    b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        i. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
        ii. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        iii. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must

be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).

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

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

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

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

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

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

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

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

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

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

B. Special Practices

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

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

3. The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-3 containment.

4. A system is established for reporting and documenting near misses, laboratory accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.

5. Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the laboratory supervisor, institutional management, and appropriate safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.

6. Biological materials that require BSL-3 containment are placed in a durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the laboratory. Once removed, the primary container is opened within a BSC in BSL-3 containment unless a validated inactivation method is used. See Appendix K. The inactivation method is documented in-house with viability testing data to support the method.

7. All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. No work with open vessels is conducted on the bench. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of personal protective equipment and other administrative and/or engineering controls, such as centrifuge safety cups or sealed rotors, are used, based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.

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

    a. Equipment or material that might be damaged by high temperatures or steam is decontaminated using an effective and verified method, such as a gaseous or vapor method.

9. A method for decontaminating all laboratory waste is available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

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

11. Decontamination processes are verified on a routine basis.

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

1. Laboratory workers wear protective clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.

2. Based on work being performed, additional PPE may be required.

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

b. Two pairs of gloves are worn when appropriate.

c. Respiratory protection is considered. Staff wearing respiratory protection are enrolled in a properly constituted respiratory protection program.

d. Shoe covers are considered.

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

D. Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building.

    a. Laboratory access is restricted. Laboratory doors are lockable in accordance with institutional policies. Access to the laboratory is through two consecutive self-closing doors. A clothing change room and/or an anteroom may be included in the passageway between the two self-closing doors.

2. Laboratories have a sink for handwashing. The sink is hands-free or automatically operated and should be located near the exit door. If a laboratory suite is segregated into different zones, a sink is also available for handwashing in each zone.

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

4. The laboratory is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping.

    a. Carpets and rugs are not permitted.

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

    c. Seams, floors, walls, and ceiling surfaces are sealed. Spaces around doors and ventilation openings are capable of being sealed to facilitate space decontamination.

    d. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases.

    e. Walls and ceilings are constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

5. Laboratory furniture can support anticipated loads and uses.

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

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

6. All windows in the laboratory are sealed.

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

8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. See Appendix A, Figure 11. Filters are replaced, as needed, or are on a

replacement schedule determined by a risk assessment. Vacuum lines not protected as described are capped. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.

9. A ducted mechanical air ventilation system is required. This system provides sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory is designed such that under failure conditions the airflow will not be reversed at the containment barrier.
    a. A visual monitoring device that confirms directional airflow is provided at the laboratory entry. Audible alarms to notify personnel of airflow disruption are considered.
    b. The laboratory exhaust air is not re-circulated to any other area in the building.
    c. The laboratory exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA filtered.
10. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
    a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
    b. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.
    c. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.
    d. Class III BSCs are provided supply air in such a manner that prevents positive pressurization of the cabinet or the room.
11. Equipment that may produce infectious aerosols is used within primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters are tested annually and replaced as needed.
12. Facility is constructed to allow decontamination of the entire laboratory when there has been gross contamination of the space, significant changes in usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory is based on the risk assessment.
    a. Facility design consideration is given to means of decontaminating large pieces of equipment before removal from the laboratory.
13. Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These laboratory enhancements may include one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas-tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; containment of other piped services; or advanced access control devices, such as biometrics.

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