

Containment Feature Comparison for BSL-2, “BSL-2 with BSL-3 Practices” and BSL-3 (based on CDC BMBL, 5th ed.)

Biosafety Level 2 (BSL-2) is suitable for work involving agents that pose moderate hazards to personnel and the environment. Generally, agents handled at this level have vaccines or therapies readily available and are not an inhalation exposure risk under routine handling conditions. Because of these features, a standard lab space with procedures and equipment in place that minimize the potential for personnel to have dermal or mucous membrane contact with contaminated materials is the basis of this biosafety level. Containment emphasis is on safe sharps handling techniques and minimizing the spread of contamination to lab surfaces, including the use of a biological safety cabinet (BSC) for aerosol-generating procedures. Although newly constructed BSL-2 labs are expected to have inward airflow, escape of contaminants outside of a BSL-2 lab will most likely result from a break in defined work practices.

Biosafety level 3 (BSL-3) is applicable for clinical, diagnostic, teaching, research or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. In the instance of an “emerging agent” circumstances, the use of BSL-3 containment will generally be recommended by public health authorities until more information is established regarding the transmissibility of the infection and other agent features that would impact containment. A BSL-3 lab is designed to contain an inhalation exposure risk for personnel working in the lab as well as individuals outside the lab. This is accomplished through mechanical system controls which are intended to assure that the lab air does not enter any of the surrounding space and is highly diluted and discharged away from the building for exhaust purposes. BSL-3 lab design criteria have been expanded by USDA and other agencies to address all potential routes of pathogen escape. These additions include features such as: HEPA-filtration of lab exhaust, liquid effluent decontamination, pass-through autoclaves and decontaminant dunk tanks or chambers.

From the containment practice perspective, the differences between BSL-2 and BSL-3 are surprisingly small. The biosafety level criteria section of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) contains 4 elements. These are:

- Standard Microbiological Practices
These are “hygiene” practices intended to protect the worker and minimize surface transfer of contamination.
- Special Practices
These are administrative practices intended to inform and protect both the lab worker and persons who may come in contact with lab materials.
- Safety Equipment (Primary Barriers and Personal Protection Equipment)
These include items intended to protect the worker from exposures in the lab.
- Laboratory Facilities
These include items intended to minimize the escape of contaminants to areas outside the lab.

As they relate to BSL-2 versus BSL-3, practice differences are limited to those items in the following table.

BSL Criteria Section	BSL-2	BSL-3
Special Practice addressing BSC use	BSC (or other containment device) required for procedures likely to generate aerosols	BSC (or other physical containment device) required for all procedures involving manipulation of infectious materials
Safety Equipment addressing protective clothing	Protective lab coats, gowns, smocks, or uniforms designated for lab use must be worn while working with hazardous materials; protective clothing not to be worn outside lab; must not be taken home for laundering.	Workers in the lab wear protective clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits or coveralls; protective equipment not worn outside lab; protective clothing must be decontaminated before laundering.
Safety Equipment addressing glove limitations	Gloves need to be changed when contaminated, integrity is compromised or when otherwise necessary.	Same glove changing verbiage except an additional statement advising to wear 2 pairs of gloves when appropriate.
Safety Equipment addressing eye, face & respiratory protection	These devices should be used in rooms where infected animals are present based on risk assessment.	These devices <u>must</u> be used in such rooms.

In short, the assignment of BSL-2 with BSL-3 practices results in addition of practices to enhance the protection of the personnel working in the lab with no enhanced environmental protection unless specified and defined by risk assessment.

Understanding the requirements for achieving BSL-2 and BSL-3 containment

The following section outlines the BSL-3 criteria from the BMBL. The text in **BLACK** is applicable to both BSL-2 & BSL-3. The text in **BLUE** is a specific requirement for BSL-3.

This is provided to demonstrate that BSL-2 is largely achieved through sound lab safety practices and BSL-3 is achieved through the addition of facility features and equipment designed for containment of aerosols to a foundation of sound lab safety practices.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
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. Mouth pipetting is prohibited; 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. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
 - 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.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
- a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
 - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. (See Appendix G.)
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 childbearing 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.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2/**BSL-3** agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
 - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. 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 such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. **All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.** (BSL-2 requires use of BSC for procedures that create aerosols.)

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

1. **All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.**
2. **Workers in the laboratory wear protective laboratory 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.** (BSL-2 permits regular lab coats, not to be worn outside of lab and not to be taken home for laundering.)
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
 - a. Changes gloves when contaminated, glove integrity is compromised, or when otherwise necessary. **Wear two pairs of gloves when appropriate.**
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. **Eye, face, and respiratory protection must be used in rooms containing infected animals.** (BSL-2 requires these devices based on risk assessment.)

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors **must be self-closing** and have locks in accordance with the institutional policies. **The laboratory must be separated from areas that are open to unrestricted traffic flow within the building.** Laboratory access is restricted. **Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.**
2. Laboratories must have a sink for hand washing. **The sink must be hands-free or automatically operated.** It should be located near the exit door. **If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone.** Additional sinks may be required as determined by the risk assessment.
3. The laboratory **must** be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. **Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.**
 - a. **Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.**
 - b. **Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.**
 - c. **Ceilings should be constructed, sealed, and finished in the same general manner as walls.**

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. **All windows in the laboratory must be sealed.**
6. BSCs 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. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. **A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.**
 - a. **Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.**
 - b. **The laboratory exhaust air must not re-circulate to any other area of the building.**
 - c. **The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.**

HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

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 manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a 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 be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.**

11. A method for decontaminating all laboratory wastes should be available in the facility, **preferably within the laboratory** (e.g., autoclave, chemical disinfection, or other validated decontamination method).

12. **Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.**

13. **Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.**

14. **Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, 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; and advanced access control devices, such as biometrics.**

15. **The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.**