

## BSL-3 Criteria

Biosafety Level 3 (BSL3) is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents, which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets or other physical containment devices, or wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that some existing facilities may not have all the facility features recommended for BSL3 (i.e., double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.), may be achieved in a Biosafety Level 2 facility, providing: 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for BSL3 are rigorously followed. The decision to implement this modification of BSL3 recommendations should be made by the PI and approved by the Biological Safety Committee and verified by the Biological Safety Office.

The following standard and special safety practices, equipment and facilities apply to agents assigned to BSL3:

### Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the Principal Investigator (PI) when experiments or work with cultures and specimens are in progress.
- Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted. (See [Biohazardous Waste](#)).
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility

- A [biohazard sign](#) must be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name and phone number of the investigator.
- An insect and rodent control program is in effect.

### Special Practices

- Laboratory doors are kept closed when experiments are in progress.
- The PI controls access to the laboratory and restrict access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may have serious consequences are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The PI has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.
- The PI establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
- When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the PI or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.
- Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.
- A biosafety manual specific to the laboratory is prepared or adopted by the PI and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.
- The PI is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the PI or other competent scientist proficient in safe microbiological practices and techniques.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
  - Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or

aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Syringes that re-sheath the needle, needleless systems, and other safe devices are used when appropriate.
- Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.
- All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.
- Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.
  - Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.
  - Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.
- Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.
- Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
- Animals and plants not related to the work being conducted are not permitted in the laboratory.

#### Safety Equipment (Primary Barriers)

- Protective laboratory clothing, such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.

- Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
- Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
- All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonic eggs, etc., are conducted in a Class II or Class III [biological safety cabinet](#) (BSC).
- When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety buckets or sealed rotors) are used.
- Respiratory and face protections are used when in rooms containing infected animals.

#### Laboratory Facilities (Secondary Barriers)

- The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable. A clothes-changing room may be included in the passageway.
- Each laboratory room contains a sink for hand washing. The sink is hands-free or automatically operated and is located near the room exit door.
- The interior surfaces of walls, floors, and ceilings of areas where BSL3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of coved floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed. Openings such as around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.
- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- All windows in the laboratory are closed and sealed.
- A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfectant, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.
- BSCs are required and are located away from doors, from room supply louvers, and from heavily traveled laboratory areas.
- A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust

air is not re-circulated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.

- HEPA-filtered exhaust air from a Class II BSC can be re-circulated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II BSC is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III BSC are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets.
- Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
- Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).
- An eyewash station is readily available inside the laboratory.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- The BSL3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.
- Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.