Basics of Biosafety Level 2

Biosafety Level 2 is more restrictive than BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is definitely limited when work is being conducted, (3) extreme precautions are taken with contaminated sharp items, and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

BSL-2 Standard Microbiological Practices

1. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

2. Eating, drinking, smoking, handling contact lenses, and applying cosmetics 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 for this purpose only.

3. Mouth pipetting is prohibited; mechanical pipetting devices are used.

4. Policies for safe handling of sharps (when non-sharps are not available) are instituted.

5. All procedures are performed carefully to minimize the creation of splashes or aerosols.

6. Work surfaces are decontaminated at least once a day and after any spill of viable material.

7. 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 at off-site locations from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

8. An integrated pest management control program is in effect.

BSL-2 Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous 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 laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.

3. When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., immunization), a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. When deemed appropriate by OSEM/OHSD, and considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

6. A LSP with these biosafety provisions is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

7. Laboratory 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 or policy changes.

8. 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. Plastic ware 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 which re-sheathe the needle, needle-less systems, and other safe devices should be 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 are decontaminated before disposal, according to any local, state, or federal regulations.
9. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. Spills and accidents which result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

12. Animals not involved in the work being performed are not permitted in the lab.

**BSL-2 Safety Equipment (Primary Barriers)**

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
   - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
   - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guards) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the biosafety cabinet.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed. Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and are not worn outside the laboratory. Disposable gloves are not washed or reused.
BSL-2 Laboratory Facilities (Secondary Barriers)

1. Each laboratory contains a sink for hand washing.

2. The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.

3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

5. If the laboratory has windows that open, they are fitted with fly screens.

6. A method for decontamination of infectious or regulated laboratory wastes is available (e.g., autoclave, chemical disinfection, incinerator, or other approved decontamination system).

7. An eyewash facility is readily available.

8. The laboratory should be at negative pressure with respect to areas outside the lab. Hoods and biosafety cabinets should be positioned away from doors, supply vents, windows, heavy traffic patterns and other cross drafts.