Biosafety Levels – Quick Tips

When working with biological contaminants, protecting just the worker is oftentimes not enough. Systems must also be in place to protect the environment and the facility from possible contamination. Depending on the type of potentially infectious biological microorganism or laboratory animal, specific containment and safety procedures must be followed.

The Centers for Disease Control and Prevention (CDC) in conjunction with the National Institutes of Health (NIH) have defined four biosafety levels, BSL-1 through BSL-4. Within each level, laboratory practices and techniques; laboratory facilities; and engineering controls have been recommended for the handling of hazards posed by the infectious organisms within each biosafety level. The following is a summary of recommended biosafety levels for infectious agents.

Biosafety Level 1 (BSL-1)

Classification:

Biosafety Level 1 (BSL-1) laboratories are working with well-characterized agents that are not known for causing disease in healthy adult humans and are of minimal potential hazard to laboratory personnel and the environment.

Standard 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.
6. All procedures must be performed so as to minimize the creation of splashes and/or aerosols.

7. Work surfaces must be decontaminated with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.
8. All cultures, stocks and other potentially infectious materials must be decontaminated before disposal.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to help prevent exposures and exposure evaluation procedures.

Special practices:

None.

Safety equipment (primary barriers):

Biological safety cabinets (BSC) are generally not used with this level of agent. Employees are encouraged to wear lab coats, gowns or uniforms to help prevent contamination of street clothes. Gloves should be worn if skin is broken or a rash is present. Appropriate eyewear should be worn for procedures where splash of microorganisms or hazardous materials is anticipated.

Laboratory facilities (secondary barriers):

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets and equipment should be accessible for cleaning.
    1. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals.
    2. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior should be fitted with screens.

Biosafety Level 2 (BSL-2)

Classification:

BSL-2 laboratories are working with agents of moderate potential hazard to personnel and the environment. Laboratory personnel have specific training in handling pathogenic agents.

Standard practices:

Same as BSL-1.

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, as appropriate and offered available 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 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.
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 that may generate an aerosol should be conducted within a biological safety cabinet (BSC) or other physical containment devices.

Safety equipment (primary barriers):

1. Properly maintained BSCs, other appropriate personal protective equipment (PPE) or other physical containment devices must be used whenever:
    1. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally and harvesting infected tissues from animals or eggs.
    2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks or uniforms designated for laboratory use must be worn while working with hazardous materials. Protective clothing must be removed before leaving for non-laboratory areas. Protective clothing must be disposed of appropriately, or deposited for laundering by the institution. It is suggested that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, faceshield or other splatter guard) is to be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should 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.
5. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
    1. Change gloves when contaminated, glove integrity is compromised or when otherwise necessary.
    2. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

- Not wash or reuse disposable gloves. Used gloves must be disposed of with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

6. Eye, face and respiratory protection should be used in rooms containing infected

animals as determined by the risk assessment.

Laboratory facilities (secondary barriers):

Same as BSL -1 plus:

1. Biological safety cabinets (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, windows that can be opened, heavily traveled laboratory areas and other possible airflow disruptions.
2. Vacuum lines should be protected with liquid disinfectant traps.
3. An eyewash station must be readily available.
4. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
5. High efficiency particulate air (HEPA) filtered exhaust air from a Class II BSC can be safely recirculated back 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.
6. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration or other validated decontamination method).

Biosafety Level 3 (BSL-3)

Classification:

Laboratories that fall under this category include clinical, diagnostic, teaching, research or production facilities working with indigenous or exotic agents that may cause serious or potentially lethal diseases as a result of exposure by inhalation.

Standard practices:

Same as BSL-1 and BSL-2.

Special practices:

Same as BSL-2 plus:

1. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
2. Controlled access required.
3. All waste must be decontaminated.
4. Laboratory clothing must be decontaminated before laundering.

Safety equipment (primary barriers):

1. All procedures involving the manipulation of infectious materials must be conducted within a biological safety cabinet (BSC) (preferably Class II or Class III) or other physical containment devices.
2. Workers in the laboratory must wear protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits or coveralls. Protective clothing must not be worn outside of the laboratory. Reusable clothing must be decontaminated before being laundered. Clothing must be changed when contaminated.
3. Eye and face protection (goggles, mask, faceshield or other splash guard) must be 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 must:
    1. Change gloves when contaminated, glove integrity is compromised or when otherwise necessary. Must wear two pairs of gloves when risk assessment deems it appropriate.
    2. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

- Not wash or reuse disposable gloves. Must 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.

Laboratory facilities (secondary barriers):

Same as BSL-2 plus:

1. Physical separation from access corridors.
2. Self-closing, double-door access.
3. Exhausted air not recirculated.
4. Negative airflow into laboratory.
5. Entry through airlock or anteroom.
6. Hand washing sink near laboratory exit.

Biosafety Level 4 (BSL-4)

Classification:

This level of protection is required when working with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening diseases. Agents with a close or identical antigenic relationship to known BSL-4 agents and related agents with unknown risk of transmission are handled at this level until sufficient data is obtained to confirm work at BSL-4 or to reclassify them to a lower level.

Standard practices:

Same as BSL-1, BSL-2 and BSL-3.

Special practices:

Same as BSL-3 plus:

1. Clothing change before entering.
2. Shower on exit.
3. All material decontaminated on exit from facility.

Safety equipment (primary barriers):

All procedures within these facilities are conducted in a Class III BSC or in a Class II BSC used in conjunction with full-body, air-supplied, positive pressure suits.

Laboratory facilities (secondary barriers):

There are two models for BSL-4 laboratories and they can follow requirements of either model or a combination of the two:

1. Class III Cabinet laboratory – Handling of agents must be done in a Class III BSC. Exhaust air from the Class III BSC must pass through two HEPA filters prior to release to external environment.
2. Suit laboratory – Dedicated room air supply and exhaust systems are required. A one-piece positive pressure supplied air protective suit must be worn by the laboratory personnel.

BSL-4 builds upon BSL-1, -2 and -3. Here are a few different criteria that must be fulfilled in a BSL-4 laboratory:

1. Entry must be limited by a secure, locked door. A means of documentation of persons entering and leaving the laboratory must be maintained.
2. No one should work alone in the laboratory—a two-person rule should apply.
3. An inner disposable pair of gloves must be worn with an outer pair of gloves.
4. A method of communication for emergency contacts must be developed between the staff working in the laboratory.
5. There must be a controlled air system—negative pressure must be maintained in the laboratory.
6. A double-door, pass-through autoclave must be readily available for the materials passing out from Class III BSC.
7. A chemical shower must be provided for the decontamination of the positive-pressure suit before the staff leaves the laboratory.
8. A separate detailed work manual and an emergency program should be developed.

Additional details may be found in the U.S. Department of Health and Human Services Public Health Service Centers for Disease Control and Prevention National Institutes of Health's Biosafety in Microbiological and Biomedical Laboratories 5th Edition on pages 51– 58.

Sources

For other documents related to this topic, see:

Quick Tips #206: Disinfectants and Antiseptics
OSHA Lab Standard, 29 CFR 1910.1450.
Biosafety in Microbiological and Biomedical Laboratories (BMBL) 4th Edition.

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