Chapter 19

Biosafety and Biohazards: Understanding Biosafety Levels and Meeting Safety Requirements of a Biobank

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Abstract

When it comes to biobanking and working with different types of laboratory specimens, it is important to understand potential biohazards to ensure safety of the operator and laboratory personnel. Biological safety levels (BSL) are a series of designations used to inform laboratory personnel about the level of biohazardous risks in a laboratory setting. There are a total of four levels ranked in order of increasing risk as stipulated by the Center of Disease Control and Prevention (CDC) (Biosafety in microbiological and biomedical laboratories, 5th edn. HHS publication no. (CDC) 21-1112. https://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf. Accessed 2 Jan 2016, 2009). We will address the main distinctions between these levels including briefly introducing hazards characteristics that classify biohazardous agents, as well as define the essentials in meeting safety requirements.

Key words Biosafety, Safety requirements, Biohazard, Biosafety levels, Personal protective equipment

1 Introduction

BSLs are a series of safety precautions that will help reduce laboratory personnel's risk of exposure to potentially infectious biohazardous agents. There are four biosafety levels that are implemented and defined by the CDC. Each biosafety level has specific containment controls, which include microbiological practices, safety equipment, and facility safeguards to protect laboratory workers, the public and the environment from exposure to infectious biohazards that are used in the lab. These containment controls build on the preceding level of safety, in a pyramid-like fashion, as the risk level increases. Biosafety levels dictate the type of work practices that are allowed to occur in a lab setting and play a huge role in the design of the facility.

1.1 Biohazardous Agents Risk Assessment

Risk assessment plays an important role in determining the biosafety level of a lab. The CDC defines risk assessment as the process by which the appropriate selection of practices and safe guards

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respective of the agents are implemented to prevent laboratory-associated infections. Risk assessment is bound by two main categories: agent hazards and laboratory procedure hazards. Similarly, the main determinants of biosafety levels are dependent on the work performed in the laboratory as well as the agents used. Mainly, the following parameters are considered during the risk assessment process:

1. Infectivity—the ability of a pathogen to establish an infection or a pathogen's capacity for horizontal transmission,
2. Transmissibility, and
3. Nature of work conducted.

Specifically, when investigating biohazardous agents that will be handled and manipulated by the laboratory, risk assessment involves scrutinizing the principal hazardous characteristics of an agent. These include:

1. Capability to infect and cause disease in a susceptible human or animal host.
2. Virulence as measured by the severity of the resulting disease.
3. The availability of preventive measures and effective treatments for disease
4. Additional characteristics of hazardous agents include route of transmission of laboratory infection, infective dose, stability of agent in the environment, host range, and its endemic nature.

All these factors contribute to the respective agent's risk assessment. The World Health Organization (WHO) has established a risk group classification for hazardous agents used in a biomedical setting [1]. These agents are stratified mainly based on the route of transmission of the natural disease. It is important to note that these four risk group classifications do not equate to biosafety levels implemented in a laboratory setting.

2 Biosafety Level Distinctions

There are four biosafety levels that are implemented and defined by the CDC. Biosafety levels are an important and integral part of biohazardous communication and training for work in these facilities. Most institutions have biosafety review boards and committees that ensure that these guidelines are followed and can often address laboratory-specific questions. Clinical work involving human specimens are generally characterized under BSL2 guidelines, though oftentimes the infectious natures of clinical specimens are unknown. BSL2 level of compliance aligns well with Occupational Safety and Health Administration (OSHA) (the oversight

body for enforcement of safety and health legislation) standard when working with specimens that contain blood or blood traces [2]. Strict adherence to guidelines and suggestions given by the CDC will help communicate a safer workspace and promote compliance in the laboratory. This chapter addresses biosafety level distinction and classification in a standard laboratory but does not address the specific guidelines that are given for vertebrate animal biosafety level criteria. A thorough and comprehensive exploration of biosafety levels, safety practices, and regulatory standards for animal biosafety levels could be found in the CDC's Biosafety in Microbiological and Biomedical Laboratories [3].

2.1 Biosafety Level 1

Biosafety level 1 (BSL1) is the lowest risk level and involves work and procedures performed with established and characterized strains of microbes that are not known to consistently cause disease in healthy adult humans. These agents generally pose minimal threat to environment. Examples of these microbes include: *Bacillus subtilis*, *Naegleria gruberi*, *S. cerevisiae*, and *E. coli*. Research conducted in BSL1 laboratories is generally performed on open laboratory benches without the need for special containment. The CDC advises standard microbiological practices to be followed which are described below.

2.1.1 BSL1 Procedures and Practices

1. Hand washing is required after working/handling potentially hazardous materials and before leaving the laboratory
2. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing of food are not permitted in laboratory areas. Refrigerators and freezers used in the laboratories must be clearly labeled for laboratory use only.
3. Mouth pipetting is prohibited.
4. Reduced use of sharps, such as glass Pasteur pipettes, needles, and scalpels, is advised including the implementation of engineering controls and proper use of sharps needles (no recapping of needles, disposal of used needles in puncture-proof containers etc.).
5. Minimize the creation of splashes and aerosols.
6. Decontamination of work surfaces after work with microbial/other BSL1 designated agents.

2.1.2 Safety Equipment for BSL1

BSL1 labs do not require special containment equipment like biological safety cabinets. The following are a list of primary barriers and personal protective equipment that are used in a BSL1 setting:

1. General laboratory coats are recommended.
2. Use of protective eyewear is required when conducting procedures that could potential create aerosols and splashes of hazardous materials.

3. Gloves should be worn when handling hazardous or potentially hazardous materials to protect hands from exposure. Gloves should be changed if contaminated or punctured. Gloves should not be reused, and alternatives to latex should be available in case laboratory personnel have latex allergies. Hands should always be washed prior to leaving the laboratory.

2.1.3 Laboratory Facilities for BSL1

1. Available sink for hand washing.
2. Doors should be available to separate workspace from the rest of the facility and to provide access control.
3. The laboratory bench should be resistant to water, heat, organic solvents, acids, and bases.
4. Laboratory chairs should be made entirely of nonporous material and can be easily cleaned and decontaminated.

2.2 Biosafety Level 2

Biosafety level 2 (BSL2) builds on the safety precautions and procedures of BSL1. Biohazardous agents that are under BSL2 pose moderate hazards to the environment and to laboratory personnel if accidentally exposed by skin contact, inhalation, or ingestion. Examples of BSL2 hazardous agent are *Staphylococcus aureus*, *Salmonella*, and human cell lines. BSL2 labs differ from BSL1 lab by the additional necessary training specific for handling BSL2 pathogenic agents. These laboratories also have restricted access to workspaces where BSL2 hazardous agents are handled, used, and manipulated. Lastly, all procedures where infectious or possibly infectious aerosols/splashes could be created are conducted in biological safety cabinets (BSC).

2.2.1 BSL2 Procedures and Practices

1. Access to laboratory should be restricted when work is being conducted using BSL2 hazardous agents.
2. Proper warning signs regarding the potential hazards should be evident to everyone entering the laboratory.
3. Laboratory personnel should be properly trained in handling BSL2 agents
4. Laboratory personnel should be offered immunizations for agents that are handled in the laboratory.
5. A laboratory specific biosafety manual must be prepared, implemented and easily accessible.
6. Infectious and potentially infectious materials should be placed in a durable, leakproof container during collection, handling, processing, storage and transport.
7. Laboratory equipment should be routinely decontaminated after spills, splashes etc.
8. Spills involving infectious materials should be contained and decontaminated by properly trained personnel.

9. Equipment exposed to infectious agents should be cleaned and decontaminated before removal from the laboratory for any occasion.
10. All procedures involving the handling and manipulation of BSL2 agents should be conducted in a BSC or other physical containment devices.
11. Animals and plants not associated with the work performed will not be permitted in the laboratory.

2.2.2 Safety Equipment for BSL2

BSL2 laboratories include all of the safety equipment and precautions used for BSL1 labs. This includes all engineering controls, safety equipment, and any special laboratory facilities. The following are a list of primary barriers and personal protective equipment that are used in a BSL2 setting.

1. Special containment equipment like biological safety cabinets must be used for procedures where infectious aerosols could potentially be created including pipetting, centrifuging, grinding, blending, shaking, sonicating, or general handling of open containers containing infectious materials.
2. Protective laboratory coats and gowns must be worn at all times while working with hazardous materials and removal of protective clothing must be done before leaving the laboratory.
3. Eye and face protection (i.e., goggles, face shield, mask) should be used for any anticipated splashes, sprays, and other possible risk exposure.

2.2.3 Laboratory Facilities for BSL2

1. Laboratory doors should be self-closing and only grant restricted access to authorized personnel while work is being conducted.
2. The design of these laboratories should facilitate easy cleaning and decontamination.
3. Absorbent floor coverings like rugs and carpets are not permitted.
4. Windows that open to the exterior are not recommended; however, if they exist, they should be fitted with screens and sealed.
5. Installed BSCs should be placed so as they do not interfere with the room's air supply and exhaust and are a sufficient distance away from doors and heavily occupied areas of the laboratory.
6. BSCs should be certified annually. HEPA filter exhausted air from a Class II BSC can be safely recirculated back into the lab.
7. Eyewash stations should be readily available.

8. An autoclaving facility or another method of laboratory waste decontamination should be readily accessible and available (i.e., incinerator).
9. Vacuum lines should be primed and protected with liquid disinfectant traps.

2.3 Biosafety Level 3

Biosafety Level 3 (BSL3) builds on the safety precautions and procedures of BSL1 & 2. This includes all engineering controls, safety equipment, and any special laboratory facilities. Biohazardous agents that are under BSL3 are indigenous, exotic and may cause serious or lethal disease through respiratory transmission. Examples of BSL3 hazardous agents are M*ycobacterium tuberculosis,* SARS coronavirus, *Chlamydia psittaci*, etc. BSL3 labs differ from BSL2 labs by the nature of transmission of hazardous agents. BSL3 labs also require the added precaution of directional airflow (negative air flow) to ensure that air flows from nonlaboratory areas into laboratory areas.

2.3.1 BSL3 Procedures and Practices

1. Laboratory personnel must receive specific training in handling and manipulation of BSL3 agents, which can be potentially lethal.
2. All procedures involving the handling and manipulation of such agents must be performed in a BSC or other physical containment device.
3. Materials that need to be decontaminated outside of the immediate laboratory need to be transported in a leakproof, secure, and durable container.
4. The universal biohazard symbol must be visibly placed on doors at the laboratory entrance.
5. Exposures to infectious materials must be evaluated immediately and procedures described in the laboratory biosafety manual must be followed. All incidents must be reported to the laboratory supervisor and lab records must be maintained.

2.3.2 Safety Equipment for BSL3

1. All procedures conducted in a BSL3 laboratory that involves the handling of infectious material must be conducted using a Class II/Class III biosafety cabinet.
2. Person protective equipment (PPE) includes a solid front with a tie-back laboratory attire or wrap around gowns, scrub suits or coveralls. The PPE worn in BSL3 laboratories should not be worn outside the lab at any time. Some BSL3 PPEs may be reusable, but must be decontaminated before they are laundered.
3. In rooms containing infected animals, eye, face, and respiratory protection must be used.

2.3.3 Laboratory Facilities for BSL3

1. Laboratory access is restricted only to authorized and trained personnel.
2. BSL3 laboratories must be accessed through two separate self-closing and locking doors. An anteroom, where clothing may be changed, and proper PPE adorned, is suggested to be situated in the passageway between the two self-closing doors.
3. The laboratory must be designed with minimal horizontal surfaces so that it can be easily cleaned and decontaminated.
4. All crevices in the floor, wall, ceiling, doors, ventilation openings, and surfaces should be sealed.
5. Ceilings and walls should have a smooth finish and all surfaces should be easy to clean and decontaminate.
6. Floors must be slip-resistant, waterproof, and resistant to chemical. The installation of seamless, sealed, resilient floors should be considered.
7. The entire laboratory must be decontaminated in case there are major renovations, maintenance, shut downs, or any other significant changes to the laboratory space.
8. All vacuum lines must be protected with HEPA filters in addition to liquid disinfectant in the traps.
9. BSL3 laboratories must have ducted air ventilation systems. This system provides sustained directional airflow by drawing air from clean areas into the laboratory and moving it toward potentially contaminated areas. The space should be designed such that under conditions of failure, the airflow will not be reversed. Some pointers to remember about the ventilation system are:
   (a) Laboratory personnel should be able to easily identify the direction of airflow.
   (b) A visual monitoring device indicating the directional airflow should be placed at the entrance of the laboratory. Audible alarms to notify airflow disruption are preferred.
   (c) Laboratory air exhaust should not recirculate to any other areas of the building
10. HEPA filter housing should have gas-tight isolation dampers, decontamination ports and/or bag-in/bag-out capability (with the appropriate decontamination procedures). The filters and the housing should allow for leak testing of each filter and should be tested and certified annually.
11. HEPA filtered exhaust air from a Class II BSC can be safely recirculated into the laboratory environment only if they are recertified and tested annually and used under manufacturer's recommendations. BSCs can be directly exhausted outside

through a hard connection or connected to the laboratory's exhaust system.

12. Class III BSCs must be hard connected to the cabinet's second exhaust HEPA filter.
13. Supply air for BSCs must be maintained so that negative pressurization of the cabinet is maintained.
14. Containment devices that contain HEPA filtration (i.e., BSCs) will serve as primary barrier devices for equipment that have the potential to produce infectious aerosols. HEPA filters must be tested and replaced annually.
15. Laboratory enhancements may be required based on risk assessment of the BSL3 laboratory. Such enhancements may include: an anteroom for clean storage of equipment, supplies and dress-in, shower-out capabilities; gas tight dampers for laboratory isolation; final HEPA filtration of laboratory exhaust air in addition to HEPA filters already installed on containment devices; laboratory effluent decontamination; and advanced access control devices (i.e., biometrics).
16. Facility design, operational procedures and parameters must be documented prior to full operation of BSL3 laboratory. The entire facility must be reverified and documented annually.

2.4 Biosafety Level 4

Biosafety Level 4 (BSL4) laboratories are the highest level of biological safety, and are very rare. They are usually separate facilities that are physically disconnected from other facilities and sufficiently isolated. They build on the safety precautions and procedures of BSL1, 2, and 3 laboratories. This includes all engineering controls, safety equipment, and any special laboratory facilities. Biohazardous agents that are under BSL4 are dangerous and exotic and pose a high risk through aerosol/respiratory transmission in the laboratory that can lead to life-threatening disease and are lethal. Vaccines and treatments are generally not available for these agents. Some investigated agents in which routes of transmission remain unclear are given BSL4 designation. Examples of BSL4 hazardous agents include Ebola, Marburg, and Lassa Viruses and Crimean-Congo hemorrhagic fever. BSL4 labs differ from BSL3 labs by the specific training required by laboratory personnel and staff in handling extremely hazardous infectious agents. This is inclusive of primary and secondary containment and all standard and special practices that involve handling, manipulation, and storage of these dangerous BSL4 agents. BSL4 laboratories are divided into two types, cabinet laboratories and suit laboratories. The differences between the two as well as addition BSL4 specific protective measures will be discussed in detail below.

2.4.1 BSL4 Procedures and Practices

1. Only people whose presence is required for scientific or other necessary support purpose are authorized to enter BSL4 laboratory spaces.
2. All entries into the facility must be properly logged including date, time, and names of all persons entering and leaving the laboratory.
3. Laboratory personnel must change clothing before entering BSL4 laboratory space.
4. Laboratory personnel must shower before exiting BSL4 space.
5. Entry and exit of laboratory must be done through these specific clothing changing rooms except in the event of an emergency.
6. Used laboratory clothing must be treated as contaminated materials and should not be removed from the inner changing room through the personal shower. They should be thoroughly decontaminated before laundering.
7. All materials exiting BSL4 laboratories must be thoroughly decontaminated.
8. It is crucial that laboratory supervisors are responsible for oversight over laboratory personnel, this includes ensuring that laboratory personnel:
   (a) Receive appropriate training for specific operation and procedures of the respective BSL4 facility.
   (b) Demonstrate high proficiency in standard and special practices when working with BSL4 containment and hazardous agents.
   (c) Receive necessary annual updates and are given additional training when any procedural or policy changes occur.
9. Biological materials that are removed from the laboratory must be transferred in a nonbreakable, sealed primary container and then further enclosed in a nonbreakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or a decontamination shower. Once removed from the laboratory, packaged material must remain viable and intact and must not be opened outside of BSL4 containment unless inactivated by a validated method.
10. Supplies, equipment, and materials not brought in to the BSL4 laboratory through the changing room must be brought through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel within the laboratory may retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. The doors of the fumigation chamber or autoclave must be locked in a manner that prevents opening of the

outer door unless the autoclave or fumigation chamber has completed a decontamination cycle.

11. Only necessary equipment and supplies should be stored inside the BSL4 laboratory.
12. Emergency procedures and protocols must be established and include plans for medical emergencies, facility malfunctions, escaped animals, and other emergencies. Training for emergency procedures must be provided to all laboratory staff.

2.4.2 Safety Equipment for BSL4

Cabinet laboratory: All work involving infectious materials in the laboratory must be conducted in a Class III BSC. Specific class III BSC protocols include:

(a) Decontaminated materials passing out of Class III BSCs must go through a double-door and pass through autoclaves. Autoclave doors must be interlocked so they only one can be opened at any given time and be automatically controlled so that the outer autoclave door can be opened after the decontamination cycle has been completed.

(b) Class III BSC must have passed through dunk tank, fumigation chamber, or equivalent decontamination methods so materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.

(c) HEPA filters must be placed on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

(d) The interior of the BSC must be have a smooth finish for easy cleaning and decontamination; all sharp edges must be eliminated to reduce the risk of cuts and glove tears. All equipment in the BSC should also be free of sharp edges.

(e) Class III cabinet gloves must be inspected prior to use and changed if necessary if damaged. Gloves should be replaced annually when cabinet is recertified.

(f) No personal clothing, jewelry or other items except eyeglasses are allowed in the BSL4 past the shower area.

(g) Disposable gloves are required underneath cabinet gloves for added protection. Gloves cannot be worn outside of the laboratory

Suit laboratory: All work conducted in a BSL4 suit laboratory must be conducted in a one-piece positive pressure supplied air suit.

(a) All manipulations and handling of infectious agents must be performed within a primary barrier system (i.e., BSC) that is HEPA filtered. HEPA filtered air from Class II BSC can be safely recirculate back into the laboratory space.

(b) Laboratory personnel must wear protective laboratory clothing like scrub suits before entering the room for donning positive pressure suits.

(c) Disposable gloves must be worn to protect against breaks/tears in the outer suit gloves. Disposable gloves cannot be worn outside of the change area.

(d) Outer suit gloves are decontaminated during laboratory operations.

2.4.3 Laboratory Facilities for BSL4

Cabinet laboratories facility requirements:

(a) BSL4 laboratories are usually in a separate building or in an isolated and restricted zone of a shared building.

(b) The laboratory must have a dedicated supply and exhaust air, as well as vacuum lines and designated decontamination systems.

(c) Rooms in the facilities must be arranged so there is sequential passage from a dirty changing area to personal shower and out into a clean change room before exiting the BSL4 facility.

(d) The facility should have an automatically activated emergency power source to regulate the life support systems, alarms, laboratory exhaust system, lighting, entry and exit controls, BSCs, and other door gaskets in the event of emergencies.

(e) It is required for BSL4 facilities to have a double door autoclave, fumigation chamber, dunk tank, or ventilated airlock at the containment barrier for the passage of items or equipment.

(f) There must be a hands-free sink near the door of the cabinet rooms, inner change rooms and outer change room. All sinks in rooms containing Class III BSC must be connected to the wastewater decontamination system.

(g) Drains in laboratory floor must be connected directly to the liquid waste decontamination system.

(h) Plumbing or any other services that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that there is no backflow from the laboratory. Atmospheric venting systems must have two HEPA filters in series and be sealed up to the second filter.

(i) Windows must be break resistant and sealed.

(j) Central vacuum systems are not recommended, but if using such a system, it must not serve any other areas besides the room containing the cabinet.

(k) The facility should have a dedicated nonrecirculating ventilation system, and such HVAC systems can only be shared amongst similar laboratories of BSL4 designation. Laboratory must be maintained at negative pressure to surrounding areas.

(l) There must be multiple exhaust fans. It is recommended to have multiple supply fans. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

(m) A device with alarm capabilities should be used to monitor the facility's ventilation system in case there is any malfunction in the system.

(n) All supply airs to the cabinet room and exhaust air exiting the BSL4 facility must past through two HEPA filters.

(o) Fumigation chambers, dunk tanks, or equivalent decontamination methods must be provided for materials and equipment that cannot be decontaminated in the autoclave to be safely removed from the cabinet room.

(p) Liquid waste from cabinet room floor drains, sinks, and autoclave chambers within the cabinet room must be decontaminated preferably heat treatment before being discharged to the sanitary sewer. Liquid waste from showers and toilets do not require treatment.

(q) Autoclaves that open outside of the laboratory must be sealed with a bioseal that is durable, airtight, and capable of expansion and contraction. Gas discharge from the autoclave chamber must also be decontaminated.

(r) Cabinet BSL4 facilities must be reviewed and operational parameters tested and verified annually.

Suit laboratory facility requirements: They have the same requirements as cabinet laboratories and include additional provisions:

(a) BSL4 suit laboratories usually exist in a separate building or an isolated zone in a building.

(b) Rooms in facility must be arranged to ensure that directional flow through chemical shower, inner change room, personal shower, and finally outer clean changing area.

(c) BSL4 entry must be through an airlock with fitted, airtight doors.

(d) A chemical shower is required to decontaminate the surface of the positive pressure suit before worker leaves the laboratory. In the event where an emergency exit is required, or if the chemical shower system fails, gravity-fed supply of chemical disinfectant is needed.

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