Desert Research Institute

Standard Microbiological Practices for Biosafety Level 1 (BSL-1) and Level 2 (BSL-2) Laboratories

I. Introduction

Biosafety is a practice used in the proper handling of biohazardous organisms and involves implementing standard microbiological practices and principals. Because human, animal and plant materials may harbor biohazardous organisms or agents, these materials must be handled according to the hazards they present. There are four categories of biohazardous materials, therefore four levels of biosafety practices, have been implemented to control these hazards.

1. **Standard microbiological practices** are generally defined as the best safety/hygiene practices that apply to all labs that manipulate microorganisms or any biological materials that contain microorganisms. These best practices were developed to minimize the spread of contamination generated through lab processes and to protect both personnel and the environment. Although they are not regulation, they are often cited by regulatory and granting agencies such as NIH, CDC, OSHA, and USDA APHIS as minimum standards to be followed in biological research laboratories and, as such are applied to a broad spectrum of lab activities including:

- Manipulation of any microbes including bacteria, viruses, fungi, and protozoa.
- Manipulation of materials that may contain microbes including animal and plant tissues, soil samples, and water samples.
- Receiving, processing, and testing of diagnostic samples.
- Research involving recombinant DNA molecules, transgenic animals, or genetically modified plants.
- Manipulation of animals or plants that are experimentally infected with microbes.
- Work with biological toxins and other bioactive molecules.

II. Purpose and Scope

This guideline outlines the Standard Microbiological Practices that are to be followed at DRI for the conduct of work at biosafety levels 1 and 2. The information provided is extracted from the most current edition of the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), HHS Publication No. (CDC) 21-1112 Revised December 2009. The information presented reflects the minimum practices that must be in place when conducting BSL-1 and BSL- 2 research.

Biosafety Level 1 (BSL-1) is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and otherwise present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

Biosafety Level 2 (BSL-2) builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

This guideline does not contain information regarding the use of experimentally infected animals

housed in indoor research facilities, which will be discussed in a separate document, but in general, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

III. Responsibilities

The responsibility for biosafety at DRI is a team effort requiring the direct involvement of the UNR Institutional Biosafety Committee, the UNR Biosafety Officer, and the DRI Environmental Health and Safety Department (EH&S), Principal Investigators (PIs), and laboratory workers.

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A. UNR Institutional Biosafety Committee

Through a cooperative agreement with UNR, DRI utilizes the services of the UNR Institutional Biosafety Committee (IBC). The IBC is composed of at least five members that collectively represent experience and expertise in a wide range of biosafety areas applicable to UNR activities. At least two members of the IBC must be from outside the UNR community (community members not otherwise affiliated with UNR). Non-committee faculty or staff with special expertise may be asked to advise the IBC as appropriate.

The UNR IBC will, on behalf of DRI, review and subsequently approve or disapprove research protocols that involve the acquisition, use, storage, and/or disposal of biological agents proposed by DRI employees for work conducted at DRI facilities. Protocols submitted by DRI shall be approved or disapproved by the UNR IBC in accordance with its normal operating procedures. DRI EH&S will have primary responsibility for providing biosafety training to DRI researchers and for oversight of DRI projects that utilize biological agents, however the UNR IBC and/or its agents has the right to monitor activities at DRI that involve biological agents to ensure protocol compliance.

For additional information about the UNR IBC see Chapter 1 of the UNR Biosafety Manual.

B. Biological Safety Services.

DRI EH&S is an administrative unit that reports to the Executive Vice President for Research. EH&S has responsibility for development and implementation of DRI environmental health and safety policies, programs and procedures and serves the faculty and staff as a consultative resource for all matters related to occupational safety.

Through a Memorandum of Understanding and Agreement with UNR EH&S, DRI EH&S may call upon the expertise of the UNR Biosafety Officer (BSO) for assistance in biosafety compliance matters, including, but not limited to laboratory inspections to assess biosafety issues. development of biosafety protocols and emergency response plans for accidental spills and personnel contamination, and investigation of incidents involving biological agents.

C. DRI Principal Investigators

Principal Investigators (PIs) are responsible for the health and safety of all personnel in their laboratory. Specific responsibilities of the PI include:

1. Ensuring that specific laboratory hazards are effectively communicated to laboratory personnel, and that controls are in place to minimize risks associated with these hazards.
   a. Developing laboratory-specific standard operating procedures (SOPs) and protocols that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory.
   b. Ensuring that engineering controls are available, are in good working order, and are used appropriately to minimize exposure to biohazardous agents.
   c. Ensuring that appropriate personal protective equipment is available and used by laboratory personnel.
2. Ensuring that all laboratory personnel receive general biosafety training conducted by EH&S (or equivalent), as well as specific training on the hazards, procedures, and practices relevant to the laboratory they are working in. All training must be documented and records maintained.
3. Notifying the UNR IBC and obtaining prior IBC approval for work involving biohazardous material[1].
4. Ensuring that laboratory workers are provided immunizations and medical surveillance prior to exposure to biohazardous agents as appropriate (based on current recommendations of the Centers for Disease Control and Prevention and IBC recommendations).
5. Notifying DRI EH&S of any spills or incidents involving biological agents that result in exposure to laboratory personnel or the public, or release to the environment (including laboratory spills).
6. Ensuring that biological agents are disposed of in a safe, approved manner.
7. Ensuring that biohazardous materials to be transported are packaged and shipped in accordance with IATA regulations, and that persons performing these duties have appropriate and current training.
8. Ensuring that an accurate inventory of biological agents is maintained.
9. Ensuring that periodic assessments of the laboratory are conducted to self-identify health and safety weaknesses, and that identified weaknesses are remedied in a timely manner.

D. Laboratory Workers

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers are responsible for their own health and safety, as well as that of their coworkers. Specific responsibilities include:

1. Following procedures and practices established by DRI and the laboratory unit.
2. Following specified practices and procedures presented in DRI policies programs, procedures and training; and other accepted good laboratory practices to minimize exposures to biological agents, and to avoid other incidents (such as fire).

[1] All projects involving biological agents must be reviewed and approved by the Institutional Biosafety Committee (IBC) prior to commencement of the work. Principal Investigators must submit a "Memorandum of Understanding and Agreement" (MOUA) to the IBC in order to initiate the approval process.

3. Attending biosafety and other laboratory safety training as required.
4. Reporting unsafe laboratory conditions, incidents or near incidents involving personnel exposure, releases outside of containment, or other biosafety issues to the PI, EH&S, or other responsible party.
5. Utilizing control measures such as biological safety cabinets and personal protective equipment to prevent exposure to biological agents and contamination of personnel and facilities.

IV. Biosafety Level 1 (BSL-1)

The following standard practices, safety equipment, and facility requirements apply to BSL-1[2]:

A. *Standard Microbiological Practices*

1. The laboratory supervisor must implement 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 designated areas.
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 the risk of sharps injuries. The following precautions must always be taken with sharp items:
   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/syringes and other disposable sharps must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal (i.e., "sharps containers").
   c. Non-disposable sharps must be placed in a hard walled, puncture proof 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. Plasticware 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 biological material with an appropriate disinfectant. Follow the manufacturer's instructions regarding dilution ratio, contact time, and shelf life. Finally, wear the proper personal protective equipment for dispensing the disinfectant as recommended by the manufacturer.

[2] Extracted verbatim from the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition (December 2009)

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. (Generally accepted methods are summarized in Attachment 1).
    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. The sign may include the name of the agents(s) in use and the name and phone number of the laboratory supervisor or other reasonable personnel. Agent information should be posed in accordance with the institutional policy.
10. An effective integrated pest management program is required (See Appendix G of the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*)
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 infections, 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 infections. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. ***Special Practices***

None required.

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

1. Special containment devices or equipment, such as biosafety cabinets (BSCs), are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. 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. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
    a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
    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.

D. *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.
   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. Laboratories with windows that open to the exterior should be fitted with screens.

V. Biosafety Level 2 (BSL-2)

Biosafety Level 2 builds upon BSL-1 requirements. Work involving BSL-2 materials requires that all laboratory personnel have specific training in the pathogenic agents they work with and that they are supervised by scientists competent in handling infectious agents and the procedures associated with that work. In addition, access to a BSL-2 laboratory must be restricted when work is being conducted, and all procedures that have the potential to generate infectious aerosols or splashes must be conducted in biosafety cabinets or other physical containment equipment designed to control these types of exposures.

In addition to the requirements noted for BSL-1 work above, the following standard and special practices, safety equipment, and facility requirements apply to BSL-2[3].

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.

[3] Extracted verbatim from the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition (December 2009)

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. 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 of the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*)
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, 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.
    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. Animal 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 BSC or other physical containment devices.

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

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
    a. 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.
    b. 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. Remove protective clothing before leaving for non-laboratory areas, e.g., lunchrooms, restrooms, library, administrative offices, etc.). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is 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. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
   a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
   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 should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should 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. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
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, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. 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.
10. 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.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

VI. *References*

- *Biosafety in Microbiological and Biomedical Laboratories* (5th ed.). 2007. U.S. Government Printing Office, Washington, D.C:
- *NIH Guidelines for Research Involving Recombinant DNA Molecules*. 2002.
- *OSHA Bloodborne Pathogens Standard 29CFR 1910.1030*. 2001. U.S. Department of Labor, Occupational Safety and Health Administration.
- *Biohazardous Waste Basics*. University of Tennessee Biosafety Office.

See the Biosafety section of the EH&S External Links page for updated, electronic versions of these references: http://www.dri.edu/ehs-links.

Standard Microbiological Practices Training Record

This document is intended to serve as a written record acknowledging that standard microbiological practices (SMPs) training has been given to the indicated laboratory personnel (including students) by a qualified site-specific trainer (i.e., principal investigator, laboratory manager, or other senior personnel). **Maintain a copy of this page with signatures with the SOP in Section 5 of your CHP binder**.

. **By signing below confirm that I have read and understand the Standard Microbiological Practices for Conducting Biosafety Level 1 and 2 Research and that I agree to fully adhere to these requirements"**

Last	First	nployee ID #	Signature	Date