DUQUESNE UNIVERSITY

Biosafety Guidelines

Prepared by: Environmental Health and Safety Department

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BIOSAFETY GUIDELINES

The University intends to comply with all aspects of biosafety practice presented by CDC, NIH, and US Department of Health and Human Services in the latest published editions of Biosafety in Microbiological and Biomedical Laboratories and the NIH Guidelines for Recombinant DNA and Gene Transfer. Additionally, strict adherence to standard microbiological practices and the use of Standard Precautions, defined as the assumption that all biological material contains potentially infectious agents, must be followed. The following listing of basic biosafety guidelines is considered fundamental to safe laboratory practice, but should not be viewed as comprehensive.

1. Mechanical pipetting aids shall be used when pipetting all material. Mouth pipetting is prohibited regardless of the material or manipulation.

2. Eating, drinking, storing food, handling contact lenses and applying cosmetics are not permitted in laboratory areas. Food should not be stored in refrigerators or freezers used to store biohazardous material.

3. Hands must be washed immediately after procedures involving biological material manipulation or handling, after glove removal and routinely before leaving the laboratory. All labs using biological materials must be equipped with a sink having hot and cold running water dispensed by a mixing faucet, and have soap and disposable hand towels immediately accessible.

4. Workers should decontaminate their work area following work with biological material and immediately after any spill.
5. Liquid-barrier gloves should be worn to protect faculty, staff and students from infection through contact with biological materials. Gloves must be removed prior to exiting the laboratory.

6. Procedures for the safe handling of sharps must be instituted, and efforts should be made to minimize exposure to potentially infectious material through the evaluation and use of safety-engineered sharps.

7. Laboratory coats or gowns should be worn while handling biological material. All protective equipment and laboratory garments must not be worn outside the laboratory. Laboratory clothing must be clearly labeled as potentially infectious or dirty before removal from the laboratory. If your department has opted to use a laundering service, follow those guidelines for having your lab coat laundered. If your department does not use a laundering service, your dirty lab coat will have to be thrown away and a new one purchased. Dirty lab coats should never be taken home.

8. All procedures should be performed in a manner that reduces the generation of aerosolized material. Operations such as centrifugation, sonication, and blending are known aerosol-generating procedures. Procedures or activities expected to produce potentially infectious aerosols should be performed in a certified biological safety cabinet or other equipment (fume hood) with integral engineering controls to contain aerosolized material.

**BIOSAFETY GUIDANCE FOR PRINCIPAL INVESTIGATORS**

1. The Principal Investigator (PI) is responsible for training his/her personnel on the potential hazards of the specific agents involved in the research, and the specific techniques to be used to handle the material safely. The Biosafety Officer is available for consultation and assistance.

2. The Principal Investigator is responsible for proposing the Biosafety Level (BSL) for the research/teaching project and associated handling of biological materials. The Biosafety Level is the combination of lab practices, safety equipment and laboratory facilities specifically appropriate for the operations performed, the agents handled and the laboratory function. The BSL assignment will be reviewed and approved by the Biosafety Officer and if recombinant DNA is involved, the Institutional Biosafety Committee.

2.1. The first step in Biosafety Level (BSL) determination is to investigate if a BSL or risk group has previously been assigned for the proposed biological agent.

2.1.1. Current BSL assignments can be found in the latest editions of Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition or the World Health Organization’s Laboratory Biosafety Manual.
2.1.2. These BSLs were assigned assuming activities typically associated with the growth and manipulations of the quantities and concentrations of infectious agents required to accomplish identification or typing. If the protocol requires higher concentrations, larger volumes, or practices likely to endanger personnel, the BSL assignment may be increased.

2.2 For purposes of recombinant DNA, risk groups are assigned based on the relative pathogenicity of an agent to healthy adults. Most often, risk group assignments equate to BSL, i.e. agents in risk group 2 are assigned to BSL-2. Risk Group assignments can be found in the Guidelines for Research Involving Recombinant DNA Molecules, NIH Guidelines. [https://osp.od.nih.gov/biotechnology/nih-guidelines/](https://osp.od.nih.gov/biotechnology/nih-guidelines/)

2.2.1 These BSLs were assigned assuming activities typically associated with the growth and manipulations of the quantities and concentrations of infectious agents required to accomplish identification or typing. If the protocol requires higher concentrations, larger volumes, or practices likely to endanger personnel, the BSL assignment may be increased.

2.2.2 Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the risk group assigned to the parent strain.

2.3 If the biological agent is not listed in these references, then the PI must assign a BSL using the best available information. Assistance in assigning BSLs is available from the Biosafety Officer, the Institutional Biosafety Committee and the Biohazards Committee.

**BIOSAFETY LEVELS**

There are four internationally accepted biosafety levels. These levels represent a combination of laboratory practices, techniques, protective equipment and facility features.

1. Biosafety Level 1 (BSL1)

   1.1. BSL1 agents are viable microorganisms not known to cause disease in healthy adults. BSL agents are recommended for undergraduate teaching laboratories and for work performed on open laboratory benches.

   1.2. BSL1 practices and facilities represent a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers.

   1.3. A sink for hand washing is required within the laboratory. Special containment equipment is not required for manipulations of BSL1 agents.

   1.4. Biohazard warning signs indicating BSL1 must be posted at each entrance to the lab.
2. Biosafety Level 2 (BSL2)

2.1. BSL2 agents are moderate-risk, viable microorganisms associated with human diseases of varying severity in healthy adults. These agents can be hazardous through various exposure routes, but not inhalation.

2.2. BSL2 is necessary when work is done using human-derived blood, body fluid, or tissues and using human cell lines where the presence of an infectious agent is unknown.

2.3. BSL2 practices and techniques shall include all the standard microbiological practices used for BSL1, in addition to limiting access to the laboratory.

2.4. Biohazard warning signs must be posted at each entrance to limit access to authorized individuals, provide contact and agent information, and indicate BSL2 hazards.

2.5. Primary barriers including certified biological safety cabinets are required for aerosol-generating manipulations of agents assigned to BSL2 or tasks with splash potential of BSL2 material.

2.6. An autoclave must be accessible for sterilization of infectious waste generated in BSL2 facilities.

2.7. All BSL2 facilities must be maintained under negative pressure relative to corridors and adjacent public areas, and must have exhaust air that is not re-circulated.

3. Biosafety Level 3 (BSL3) – prohibited work at Duquesne University

3.1. BSL3 agents and materials are high-risk, viable microorganisms associated with human diseases that are potentially lethal, and are hazardous through exposures resulting from autoinoculation, ingestion, mucous membrane exposure, and particularly through inhalation.

3.2. BSL3 practices and techniques include all standard microbiological practices used in Biosafety Levels 1 and 2 in addition to limiting laboratory access to only those personnel required for the program and who have been trained in potential hazards and control measures on the specific BSL3 agent.

3.3. Operational procedures for BSL3 activity must be prepared, documented, and maintained in an operating Manual approved by the Biosafety Officer and the DUIBC. It is the responsibility of the Principal Investigator of the BSL3/ABSL3 facility to develop and maintain this Manual. Copies of the Manual will be kept by the Principle Investigator, facility director (if different from the PI), EHS and if animals are utilized, the IACUC. At a minimum, the Manual must contain the following component:

3.3.1. Approval Page signed by Principal Investigator, facility director, EHS Biosafety Officer, DUIBC Chairperson, and if animals are utilized, the IACUC Director.
3.3.2. Emergency Contact Numbers and Emergency Procedures,
- List of agents used in facility and locations of use and storage,
- Facility entrance requirements,
- List of training requirements and records of training for all current personnel,
- List of medical surveillance requirements for personnel and proof that personnel have met current requirement,
- Standard Operating Procedures and BSL3 work practices (e.g., agent handling, PPE, waste handling and disposal),
- Emergency procedures (e.g., spill, fire, personal injury),
- Autoclave verification program,
- MSDSs for hazardous chemical and infectious materials,
- Annual reverification procedures and reports of activities for same,
- Other items as deemed necessary by the DUIBC.

3.4. Appropriate personal protective equipment is required for all manipulations of agents assigned to BSL3 including lab clothing, liquid barrier gloves, respiratory protection, and safety glasses.

3.5. All personnel authorized and trained to enter BSL3 laboratories must be enrolled in University Respiratory Protection for BSL3 workers.

3.6. All manipulations of infectious agents in BSL3 facility are performed in certified biological safety cabinets.

3.7. An autoclave must be available for sterilization or infectious waste and all other materials before it is allowed to leave the BSL3 containment area.

3.8. A spill cleanup kit containing materials to contain, disinfect, and cleanup laboratory spills must be available in the BSL3 containment area.

4. Biosafety Level 4 (BSL4) – prohibited work at Duquesne University

BSL4 agents and materials are extremely high-risk, viable microorganisms associated with dangerous and exotic, life-threatening human diseases. Because of the lack of appropriate facilities and restriction by law, experiments involving agents that require BSL4 containment facilities, and/or experiments involving nonindigenous pathogens with importation, possession or use prohibitions or restrictions (as defined by Federal regulation or administrative policies) are not permitted to be undertaken on the premises of Duquesne University.
STANDARD OPERATING PROCEDURE (SOP) BIOHAZARDOUS SPILL CLEANUP

This SOP outlines the basic steps to be followed in the event of accidental release of biohazardous materials. Each Principal Investigator/laboratory director should develop specific spill cleanup methods tailored to the biohazardous agent(s), quantities, and procedures being implemented in the lab and ensure that the appropriate spill response material is kept on hand.

A Minor Biological Spill is one that the laboratory staff is capable of handling safely without the assistance of safety and emergency personnel. All other biological spills are considered major. Minor biological spills involve BSL 1 materials or releases of a small volume of BSL 2 or BSL 3 microorganisms without splashing or violent action.

**Minor Biological Spills**
- Alert people in area of spill and secure the affected area.
- Remove and disinfect any material that has been splashed on you and remove/disinfect grossly contaminated clothing.
- Don the appropriate personal protective equipment for the cleanup operation, which include at a minimum liquid barrier gloves, safety glasses, and lab coat.
- Cover the spill with paper towels or other absorbent material to absorb the spill and prevent further aerosolization.
- Pour disinfectant gently over the covered spill, working from the outside inwards.
- Wait at least 15 minutes for the disinfectant to penetrate through the contained spill.
- Using a tool (i.e. shovels, forceps) to remove the absorbent material and place in a biohazard bag for autoclaving and subsequent disposal. Do not utilize hands for broken glass or other sharps.
- Clean the original spill area with detergent to insure disinfection and cleanup.
- Notify your supervisor of the incident.
- Fill out the DU Incident/Accident Investigation Form.

A Major Biological Spill is one that requires assistance of safety and emergency personnel. Major Biological Spills involve releases of larger volumes of BSL 2 or BSL 3 microorganisms often with excessive splashing or violence or environmental (i.e. outside the lab) releases of any quantity of BSL 3 microorganisms.

**Major Biological Spill**
- Alert people in the laboratory.
- Remove and disinfect and material that has been splashed on you and remove/disinfect grossly contaminated clothing.
- Call Campus Police at x2677 (COPS).
- Have persons knowledgeable of incident and laboratory available to assist emergency personnel.
- Notify your supervisor and the Department of Environmental Health and Safety at 412-396-4763 of the incident.