



Biosafety Plan

Institutional Biosafety Committee

Program Date: March 2016



University of Scranton: Biosafety Manual

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Section 1: Introduction

1.1 Purpose and Scope

The University of Scranton (University) Institutional Biosafety Committee (IBC) has developed this Biosafety Manual to: Maintain a safe working environment by protecting persons from exposure to infectious agents and organisms containing recombinant DNA; Prevent environmental contamination; and, Comply with applicable federal/state regulations and standards. Additionally, procedures established by this plan will protect the integrity of experiments by controlling contamination. The Manual specifies protocols for the evaluation of biohazards (including those containing rDNA), design of laboratories and equipment, and work practices for limiting personnel exposure. This Manual will provide guidance in developing activity-specific Biosafety Procedures.

The Manual has been developed by the IBC for use by principal investigators (PIs) in research; However, it covers all teaching faculty, staff and students, and any non-University personnel who work in campus facilities.

Laboratory personnel defined by this plan include faculty, staff, research associates and assistants, technicians, teaching assistants, graduate and undergraduate students.

Laboratory settings under the scope of this plan include any University building where the above biohazard and rDNA laboratory operations occur.

1.2 Regulations, Standards and Guidelines and Other University Plans

The below regulations, standards and guidelines are referenced in this Manual:

U.S. Department of Labor, Occupational Safety and Health Administration (OSHA)

- Hazard Communications [HCS-2012- 29 CFR 1910.1200]
- Personal and Respiratory Protection [29 CFR Subpart I]

U.S. Centers for Disease Control and Prevention

- Biosafety in Microbiology and Biomedical Laboratories (5th Edition, 2007)

National Institutes of Health (U.S. Department of Health and Human Services)

- Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (March 2013)

This Biosafety Manual will work in concert with other Plans and Programs implemented by The University, including:

- Hazard Communication Program
- Personal/Respiratory Protective Equipment Program
- Exposure Control Plan (Bloodborne Pathogens)
- Emergency Response/Evacuation Plans
- Chemical Hygiene Plan
- Institute for Animal Care and Use Committee (IACUC)

1.3 Biohazards and Potentially Infectious Materials in the Research or Teaching Laboratory

1.3.1 Definition of Biohazards

A biohazard is an agent of biological origin that has the capacity to produce deleterious effects on humans, i.e. microorganisms, toxins, and allergens derived from those organisms; and allergens and toxins derived from higher plants and animals.

1.3.2 Routes of Exposure

Oral Infection: A variety of organisms used in the laboratory are enteric pathogens and carry the prime risk of infection by ingestion. Examples are ova and parasites, *Salmonella typhimurium*, poliovirus, and enteropathogenic *E. coli* strains.

Respiratory Route Infection: A variety of agents infect by the respiratory route. The major source of such infections is by aerosolization of biohazards. The more hazardous agents which cause respiratory infections are those which withstand drying such as *Mycobacterium tuberculosis* and *Coccidioides immitis*. Two hazards can be defined: the immediate risk from an aerosol which will be limited if the agent cannot withstand drying and the delayed risk (secondary aerosol) if the organism can withstand drying.

Puncture and Contact Infections: A variety of agents are transmitted through puncture such as arthropod-borne virus infections, protozoal infections (malaria), and human immunodeficiency virus (HIV). However, those bacterial agents which can cause septicemia also can cause infections by injection, a phenomenon particularly dangerous when a rapidly growing and pathogenic organism is injected.

Fomites: Fomites are particularly hazardous and subtle because the organisms are spread via deposition on surfaces. Careless handling of materials can lead to situations in which individuals unknowingly infect themselves by hand-to-mouth infection. The transmission of organisms from fomites to the hands and then to the mucus membranes of the eyes or nose are other examples of the route of viral infections or ingestion.

Fomites can also be created by aerosols settling on laboratory furniture, apparatus, etc. Rapid dispersal of aerosols by high air flow is an indispensable means of preventing this problem. Creation of fomites from minor spills and droplets formed during transfer of cultures is a common hazard in laboratories. The reality of this problem can readily be appreciated by transferring a dye (e.g., crystal violet) as though it were a bacterial culture. The amount of dye scattered in the work area after several such manipulations is an excellent measure of the effectiveness of containment techniques.

1.3.3 Categories of Biohazards

Categories of biohazards or potentially infectious materials include:

- Human, animal and plant pathogens: Bacteria, including those with drug resistance plasmids; Fungi; Viruses, including oncogenic viruses; *Parasites*; *Prions*.
- All human blood, blood products, tissues and certain body fluids.
- Cultured cells (all human or certain animal) and potentially infectious agents these cells may contain.

- Allergens.
- Toxins (bacterial, fungal, plant, etc.).
- Certain recombinant products.
- Clinical specimens.
- Infected animals and animal tissues.*

*When not certified to be non-infectious.

1.3.4 Recombinant DNA (rDNA)

Generation of rDNA: Experiments involving the generation of rDNA may require registration and approval by the University IBC. The NIH Guidelines¹ are the definitive reference for rDNA research in the U.S. Experiments not covered by the guidelines may require review and approval by outside agencies before initiation or funding. These experiments are not generally associated with biomedical research but are more common in the agricultural and environmental sciences.

If you have any specific questions about a particular host-vector system not covered by the guidelines, contact the Office of Biotechnology Activities (OBA), National Institutes of Health by phone (301) 496-9838, FAX (301) 496-9839 or email. Updates to the NIH Recombinant DNA Guidelines are published in the Federal Register and are available at the OBA website.

Transgenic Animals: Investigators who create transgenic animals must complete the rDNA Registration Document and submit it for IBC approval prior to initiation of experimentation. In addition, an Institutional Animal Care and Use Committee (IACUC) protocol review form must be approved.

Transgenic Plants: Experiments to genetically engineer plants by recombinant DNA methods require registration with the IBC. The NIH rDNA guidelines provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants. All plants are subject to inspection by the U.S. Department of Agriculture (USDA).

1.3.5 Other Potentially Hazardous Biological Materials in the Research Laboratory

Human Blood, Blood Products, Body Fluids, Cell Cultures and Tissues: In 1991, OSHA promulgated a standard to eliminate or minimize occupational exposure to Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. This federal regulation, "Occupational Exposure to Bloodborne Pathogens" requires a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help control the health risk to employees resulting from occupational exposure to human blood and other potentially infectious materials which may contain these or other specified agents. The University's compliance initiatives for this regulation are covered under the Exposure Control Plan maintained by the Health and Safety Office.

Animals: The use of animals in research requires compliance with the "Animal Welfare Act" and any state or local regulations covering the care or use of animals; researchers must obtain approval from the University of Scranton IACUC.

Tissue Culture/Cell Lines: When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line should be classified as the same level as that recommended for the agent.

¹<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>

Section 2: Plan Administration

2.1 Roles and Responsibilities

Roles and Responsibilities designated by this Biosafety Manual for affected University employees or employee groups are outlined below.

2.1.1	Faculty/Principal Investigator	<ul style="list-style-type: none">➤ Submit protocol to IBC for review/approval➤ Perform research and instruction➤ Ensure students/research personnel are trained and training is documented➤ Ensure accident forms are completed➤ Routinely review protocols and provide changes to IBC
2.1.2	Students	<ul style="list-style-type: none">➤ Act within one's competence level➤ Receive training on hazards and control measures➤ Request information, report concerns to PI or Course Instructor
2.1.3	Institutional Biosafety Committee	<ul style="list-style-type: none">➤ Act as a liaison for the University➤ Ensure protocols have been developed for the authorization and reauthorization of research activities➤ Review and approve research protocol and teaching submissions➤ Integrate Health and Safety elements, including Safety Checks, periodic plan review, and review of accident forms➤ Review or facilitate a review of this Manual
2.1.4	Department Administration	<ul style="list-style-type: none">➤ Ensure resources are available for the identification, evaluation and control of all biohazards and employee training➤ Ensure the working environment is acceptable for all personnel to report suggestions regarding potential improvements for employee safety

2.1.5	Other (Building Manager, Laboratory Supervisor, Facilities, etc.)	<ul style="list-style-type: none"> ➤ Facilitate contracted services (biosafety cabinets) ➤ Facilitate equipment inspections (biosafety cabinets) ➤ Facilitate work order submittals and ensure completion
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2.2 Training

All personnel covered by this Manual will be provided with training to ensure awareness of all hazards and control measures associated with their activities. Information and training sessions shall be provided for all personnel (prior to first exposing activity and routinely thereafter) who may be exposed to potential hazards in connection with biohazard/rDNA operations. This group includes faculty, students, laboratory supervisors, laboratory workers, custodial, maintenance, and stockroom personnel, and others who work adjacent to laboratories.

Records from employee training will be maintained indefinitely with this Manual.

2.3 Biohazard Warnings: Signs, Labels and Information

When biohazards are present in the work area, a hazard warning sign incorporating the universal biohazard symbol and contact information shall be posted on all access doors. Examples of the hazard warning/universal biohazard symbol are depicted in Figure 1. Additionally, postings for the below information will be provided as necessary.

- Emergency telephone numbers.
- Location signs for eyewash stations, first aid kits, fire extinguishers and exits.
- No smoking signs.
- Food and beverages prohibition.
- Chemical or other equipment hazards as designated by other applicable University programs.



2.4 Occupational Health Program

There are currently no activities permitted by The University that will require coverage under an Occupational Health Program. In the event activities under this Manual are added that necessitate coverage, an appropriate plan review will occur to include the following elements.

Immunizations for laboratory personnel covered under this plan may be required or recommended based on the scope of the activity, specifically the use of certain biohazards or animals. This designation of specific immunization protocols is not covered under this Manual due to the number of biological agents or combinations of agents that may be present in research. Specific immunizations or other occupational health-related measures that are indicated shall be determined based on the protocol review performed through the IBC.

The University's Exposure Control Plan covers the Hepatitis B vaccination requirements for employees that have a reasonable anticipated potential for exposure to blood and other potentially infectious material (bodily fluids, secretions, human tissue, etc.).

Records for any employee immunization are to be maintained with the employee's personnel file under the OSHA Medical Recordkeeping requirements.

2.5 Plan Review and Updates

The IBC shall review the entire Manual at least annually and shall make any revisions as deemed necessary to maintain compliance. The review will include any changes to regulatory requirements or guidelines, accident reports, modifications of facility equipment or operations, protocols, internal or third party safety inspections, and input from users of the Manual, where applicable.

2.6 Recordkeeping

The University will maintain accurate and complete records relative to: Manual reviews and updates; Medical* examination and consultation records; Training; Inspections; and, Accident reports.

**Records shall be maintained in accordance with 29 CFR 1910.1020(h) "Access to Employee Exposure and Medical Records".*

2.7 Safety Checks and Testing

Reviews of laboratory settings, equipment and practices shall be performed periodically by the IBC, Health and Safety or other designee. Results of any reviews shall be forwarded to the IBC/Health and Safety for review and assignment of correction action(s) as necessary.

The University's IBC requires that all BSCs be tested and certified prior to initial use, relocation, after HEPA filters are changed, and at least annually.

2.8 Permits

Importation of infectious materials, etiologic agents and vectors that may contain them is governed by federal regulation. In general, an import permit is required for any infectious agent

known to cause disease in a human. This includes but is not limited to bacteria, viruses, rickettsia, parasites, yeasts and molds. In some instances, an agent suspected of causing human disease also requires a permit.

The following vectors require import permits:

1. Animals (including birds) known or suspected of being infected with any disease transmissible to man. Importation of turtles less than 4 inches in shell length and all nonhuman primates requires an importation permit issued by the CDC, Division of Global Migration and Quarantine.
2. Biological materials: Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions or similar material, when known or suspected to be infected with disease transmissible to man.
3. Insects: Any living insect or other living arthropod, known or suspected of being infected with any disease transmissible to man. Also, if alive, any fleas, flies, lice, mites, mosquitoes or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.
4. Snails: Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either CDC or the Department of Agriculture. Any shipment of mollusks with a permit from either agency will be cleared immediately.
5. Bats: All live bats require an import permit from the CDC and the U.S. Department of Interior, Fish and Wildlife Services.

When an etiologic agent, infectious material or vector containing an infectious agent is being imported to the United States it must be accompanied by an importation permit issued by the U.S. Public Health Service (USPHS). Importation permits are issued only to the importer, who must be located in the United States. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the USPHS Division of Quarantine and release by U.S. Customs.

The importer is legally responsible to assure that foreign personnel package, label, and ship material in accordance with CDC and IATA regulations. Shipping labels, permit number, packaging instructions and the permit expiration date are also issued to the importer with the permit.

Other Permits: U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required to import or transport infectious agents of livestock and biological materials (including recombinant plants) containing animal, particularly livestock, material. Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal disease into the U. S. Applications for USDA/APHIS permits may be obtained online. Further information may be obtained by calling the USDA/APHIS at (301) 734-3277. Export of infectious materials may require a license from the Department of Commerce. Call (202) 512-1530 for further information.

Section 3: Risk Assessment

3.1 Risk Assessment Process

It is the responsibility of the Principal Investigator or Laboratory Director to conduct a Risk Assessment in order to determine the proper work practices and containment requirements for work with biohazardous material. The Risk Assessment process should identify features of microorganisms as well as host and environmental factors that influence the potential for workers to have a biohazard exposure. This responsibility cannot be shifted to inexperienced or untrained personnel. The Principal Investigator or Laboratory Director should consult with the IBC to ensure that the laboratory is in compliance with established guidelines and regulations.

When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available. Personnel performing a Risk Assessment shall use the IBC New Investigator Registration Sheet, version 9/10 (Appendix A).

3.2 Risk Assessment Considerations

Factors to consider when evaluating risk are identified below. When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available.

- **Pathogenicity:** The more severe the potentially acquired disease, the higher the risk. Salmonella, a Risk Group 2 agent, can cause diarrhea, septicemia if ingested. Treatment is available. Viruses such as Marburg, and Lassa fever cause diseases with high mortality rates. There are no vaccines or treatment available. These agents belong to Risk Group 4.
- **Route of transmission:** Agents that can be transmitted by the aerosol route have been known to cause the most laboratory-acquired infections. The greater the aerosol potential, the higher the risk of infection. Work with *Mycobacterium tuberculosis* is performed at Biosafety Level 3 because disease is acquired via the aerosol route.
- **Agent stability:** The greater the potential for an agent to survive in the environment, the higher the risk. Consider factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfections when looking at the stability of an agent.
- **Infectious dose:** Consider the amount of an infectious agent needed to cause infection in a normal person. An infectious dose can vary from one to hundreds of thousands of organisms or infectious units. An individual's immune status can also influence the infectious dose.
- **Concentration:** Consider whether the organisms are in solid tissue, viscous blood, sputum, etc., the volume of the material and the laboratory work planned (amplification of the material, sonication, centrifugation, etc.). In most instances, the risk increases as the concentration of microorganisms increases.
- **Origin:** This may refer to the geographic location (domestic or foreign), host (infected or uninfected human or animal), or nature of the source (potential zoonotic or associated with a disease outbreak).
- **Availability of data from animal studies:** If human data is not available, information on the pathogenicity, infectivity, and route of exposure from animal studies may be valuable. Use caution when translating infectivity data from one species to another.

- **Availability of an effective prophylaxis or therapeutic intervention:** Effective vaccines, if available, should be offered to laboratory personnel in advance of their handling of infectious material. However, immunization does not replace engineering controls, proper practices and procedures and the use of personal protective equipment (PPE). The availability of post-exposure prophylaxis should also be considered.
- **Medical surveillance:** Medical surveillance programs may include monitoring employee health status, participating in post-exposure management, employee counseling prior to offering vaccination, and annual physicals.
- **Experience and skill level of at-risk personnel:** Laboratory workers must become proficient in specific tasks prior to working with microorganisms. Laboratory workers may have to work with non-infectious materials to ensure they have the appropriate skill level prior to working with biohazardous materials. Laboratory workers may have to go through additional training (e.g., HIV training, BSL-3 training, etc.) before they are allowed to work with materials or in a designated facility.

Refer to the following resources to assist in your risk assessment:

- NIH Recombinant DNA Guidelines
- WHO Biosafety Manual
- CDC/NIH Biosafety in Microbiological & Biomedical Laboratories, 5th edition

3.3 Risk Group Classifications

Infectious agents may be classified into risk groups based on their relative hazard. The table below, is excerpted from the NIH Recombinant DNA Guidelines and presents the "Basis for the Classification of Biohazardous Agents by Risk Group."

Table 1 Risk Group Classification	
Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may be</i> available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)

Section 4: Containment and Control

4.1 Principles of Biosafety

The three elements of biohazard control include: (1) Laboratory practice and technique; (2) Primary Barriers, such as safety equipment; and, (3) Secondary Barriers, such as facility design.

Laboratory Practice and Technique: The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The PI or laboratory supervisor is responsible for providing or arranging for appropriate training of personnel.

Primary Barriers: The protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection. Safety equipment includes biological safety cabinets, enclosed containers (i.e. safety centrifuge cups) and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

Safety equipment also may include items for personal protection such as personal protective clothing, respirators, face shields, safety glasses or goggles. Personal protective equipment is often used in combination with other safety equipment when working with biohazardous materials. In some situations, personal protective clothing may form the primary barrier between personnel and the infectious materials.

Secondary Barriers (Facility Design): The protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of work practices, safety equipment and facility design to provide adequate containment.

Biosafety Levels (BSL) are the CDC-established levels of containment in which microbiological agents can be manipulated allowing the most protection to the worker, occupants in the building, public health and the environment. Each level of containment describes the laboratory practices, safety equipment and facility design for the corresponding level of risk associated with handling a particular agent.

Table 3 summarizes BSLs for certain infectious agents as recommended by the CDC in Biosafety in Microbiology and Biomedical Laboratories (5th Edition, 2009). The proceeding headings of this section provide further descriptions on each of the recommendations stated within the table.

Table 2				
Recommended Biosafety Levels (BSL) for Infectious Agents				
BSL	Agents	Laboratory Practices	Primary Barriers (Safety Equipment)	Secondary Barriers (Facility Design)
1	Not known to consistently cause diseases in health adults.	Standard Microbiological Practices	None required	Laboratory bench and sink required.

2	Agents associated with human disease. *Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure.	BSL-1 practice plus: *Limited access *Biohazard warning signs *Sharps precautions *Biosafety manual defining waste decontamination or medical surveillance. PPE: Lab coats; gloves; face protection	Primary barriers: Class I or II BSCs or other physical containment devices used for manipulation of agents that cause splashes or aerosols of infectious materials.	BSL-1 plus: *Autoclave available *BSL-2 Signage *Negative airflow into laboratory *Exhaust air from laboratory spaces 100% exhausted
3*	Indigenous or exotic agents with potential for aerosol Transmission. *Disease may have serious or lethal consequence	BSL-2 practice plus: *Controlled access *Decontamination of waste *Decontamination of lab clothing before laundering *Baseline serum PPE: Protective lab coats; gloves; respiratory protection as needed	Primary barriers: Class I or II BSCs or other physical containment devices used for all open manipulation of agents	BSL-2 plus: *Physical separation from access corridors *Self-closing, double-door access *Exhaust air not Recirculated *Negative airflow into laboratory
4*	Dangerous/exotic agents which pose high risk of life-threatening disease	BSL-3 practices plus: *Clothing change before entering lab *Shower on exit *Decontaminate all material on exit from facility	Primary barriers: All work conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied positive pressure personnel suit	BSL-3 plus: *Separate building or isolated zone *Dedicated supply and exhaust, vacuum, and decontamination systems

**There are currently no activities permitted by The University under the scope of this manual that involve BSL-3 or BSL-4 agents.*

4.2 Laboratory Techniques and Designated Practices

4.2.1 Hygiene

Eating, drinking, handling contact lenses, applying cosmetics (including lip balm), chewing gum, and storing food for human consumption is not allowed in the work area of the laboratory. Smoking is not permitted in any University building. Food shall not be stored in laboratory refrigerators or prepared/consumed with laboratory glassware or utensils. Break areas must be located outside the laboratory work area and physically separated by a door from the main laboratory.

Laboratory personnel must wash their hands after handling biohazardous agents or animals, after removing gloves, and before leaving the laboratory area.

4.2.2 Housekeeping

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

Laboratory personnel are responsible to clean laboratory benches, equipment and areas that require specialized technical knowledge. Laboratory staff is responsible to:

- Secure biohazardous materials at the conclusion of work.
- Keep the laboratory neat and free of clutter - surfaces should be clean and free of infrequently used chemicals, biologicals, glassware and equipment. Access to sinks, eyewashes, emergency showers and fire extinguishers must not be blocked.
- Decontaminate and discard infectious waste – do not allow it to accumulate in the laboratory.
- Dispose of old and unused chemicals promptly and properly.
- Provide a workplace that is free of physical hazards - aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, and avoidance of overloaded electrical circuits/creation of electrical hazards in wet areas.
- Remove unnecessary items on floors, under benches or in corners.
- Properly secure all compressed gas cylinders.
- Never use fume hoods or biosafety cabinets for storage.
- Practical custodial concerns include:
 - Dry sweeping and dusting that may lead to the formation of aerosols is not permitted.
 - The use of a wet or dry industrial type vacuum cleaner is prohibited to protect personnel as well as the integrity of the experiment. They are potent aerosol generators and, unless equipped with high efficiency particulate air (HEPA) filters, must not be used in the biological research laboratory. Wet and dry units with HEPA filters on the exhaust are available from a number of manufacturers.

4.2.3 Pipettes and Pipetting Aids

Pipettes are used for volumetric measurements and transfer of fluids that may contain infectious, toxic, corrosive or radioactive agents. Laboratory-associated infections have occurred from oral aspiration of infectious materials, mouth transfer via a contaminated finger and inhalation of aerosols. Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface, when cultures are mixed by pipetting, or when the last drop of an inoculum is blown out. A pipette may become a hazardous piece of equipment if improperly used. The safe pipetting techniques that follow are required to minimize the potential for exposure to biologically hazardous materials:

- Never mouth pipette. Always use a pipetting aid.
- If working with biohazardous or toxic fluid, confine pipetting operations to a biological safety cabinet.
- Always use cotton-plugged pipettes when pipetting biohazardous or toxic materials, even when safety pipetting aids are used.

- Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
- Do not forcibly expel biohazardous material out of a pipette.
- Never mix biohazardous or toxic material by suction and expulsion through a pipette.
- When pipetting, avoid accidental release of infectious droplets. Place a disinfectant soaked towel on the work surface and autoclave the towel after use.
- Use "to deliver" pipettes rather than those requiring "blowout".
- Do not discharge material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them. Do not place pipettes vertically into a cylinder. Autoclave the pan and pipettes as a unit before processing them as dirty glassware for reuse (see section D, Decontamination).
- Discard contaminated disposable pipettes in an appropriate sharps container. Autoclave the container when it is 2/3 to 3/4 full and dispose of as *infectious waste*.
- Place pans or sharps containers for contaminated pipettes inside the biological safety cabinet to minimize movement in and out of the BSC.

4.2.4 Syringes and Needles

Syringes and hypodermic needles are dangerous instruments. *The use of needles and syringes should be restricted to procedures for which there is no alternative.* Blunt cannulas should be used as alternatives to needles wherever possible (i.e., procedures such as oral or intranasal animal inoculations). Needles and syringes should never be used as a substitute for pipettes. All syringes and needle activities shall be conducted in accordance with The University Exposure Control Plan. When needles and syringes must be used, the following procedures are recommended:

- Use disposable safety-engineered needle-locking syringe units whenever possible.
- When using syringes and needles with biohazardous or potentially infectious agents, work in a biological safety cabinet whenever possible.
- Wear PPE
- Fill the syringe carefully to minimize air bubbles.
- Expel air, liquid and bubbles from the syringe vertically into a cotton pledget moistened with disinfectant.
- Do not use a syringe to mix infectious fluid forcefully.
- Do not contaminate the needle hub when filling the syringe in order to avoid transfer of infectious material to fingers.
- Wrap the needle and stopper in a cotton pledget moistened with disinfectant when removing a needle from a rubber-stoppered bottle.
- Bending, recapping, clipping or removal of needles from syringes is prohibited. If you must recap or remove a contaminated needle from a syringe, use a mechanical device (e.g. forceps) or the one-handed scoop method. The use of needle-nipping devices is prohibited (needle-nipping devices must be discarded as infectious waste).

- Use a separate pan of disinfectant for reusable syringes and needles. Do not place them in pans containing pipettes or other glassware in order to eliminate sorting later.
- Used disposable needles and syringes must be placed in appropriate sharps disposal containers and discarded as *infectious waste*.

4.2.5 Working Outside of a Biosafety Cabinet

In cases where the route of exposure for a biohazardous agent is not via inhalation (e.g., opening tubes containing blood or body fluids), work outside of a Biosafety Cabinet (BSC) may occur provided the following conditions are implemented:

- Use of a splash guard.
- Procedures that minimize the creation of aerosols.
- Additional PPE is used.

A splash guard provides a shield between the user and any activity that could splatter. An example of such a splash guard is a clear plastic panel formed to stand on its own and provide a barrier between the user and activities such as opening tubes that contain blood or other potentially infectious materials. PPE in addition to standard equipment may be assigned (e.g. face shield) for splash protection in these situations.

Needle clipping, pipetting mixing, sonication, and centrifugation can produce substantial aerosols. If you perform an aerosol generating procedure, utilize good work practice controls to mitigate potential exposures: Tightly cap tubes prior to centrifuging or vortexing; Allow aerosols to settle prior to opening tubes, equipment; Open tubes or equipment inside a containment device whenever feasible.

4.3 Safety Equipment

4.3.1 Biosafety Cabinets

Background: The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. BSCs are designed to contain aerosols generated during work with infectious material through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. All personnel must develop proficient lab technique before working with infectious materials in a BSC.

Three types of BSCs (Class I, II and III) are used in microbiological laboratories.

- The Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection. It provides protection to personnel and the environment from contaminants within the cabinet. The Class I BSC does not protect the product from "dirty" room air. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In many cases Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols that may flow back into the room.
- The Class II BSC protects the material being manipulated inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination as well as meeting requirements to protect personnel and the environment. There are four types of Class II

BSCs: Type A1, Type A2, Type B1 and Type B2. The major differences between the three types may be found in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area.

- Class II Type A1 & A2 cabinets exhaust 30% of HEPA filtered air back into the room, while the remaining 70% of HEPA filtered air flows down onto the cabinet work surface.
- Class II Type B1 & B2 cabinets are ducted to the building exhaust system. In type B1 cabinets, 70% of HEPA filtered air is exhausted through the building exhaust system, while the remaining 30% of HEPA filtered air flows down onto the cabinet work surface. Type B2 cabinets exhaust 100% of HEPA filtered clean air through the building exhaust.
- Class III cabinets are completely enclosed glove boxes that are ducted to the building exhaust system. Air from the cabinet is 100% exhausted and passes through two HEPA filters. A separate supply HEPA filter allows clean air to flow onto the work surface.

Location: Certain considerations must be met to ensure maximum effectiveness of these primary barriers. Adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance, and to ensure that the air return to the laboratory is not hindered. The ideal location for the biological safety cabinet is away from the entry (such as the rear of the laboratory away from traffic), as people walking parallel to the face of a BSC can disrupt the protective laminar flow air curtain. The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. A BSC should be located away from open windows, air supply registers, or laboratory equipment (e.g. centrifuges, vacuum pumps) that creates turbulence. Similarly, a BSC should not be located adjacent to a chemical fume hood.

Use: BSCs shall be used in accordance with standard industry practice and manufacturer recommendations. General provisions include:

- Understand how your cabinet works. The NIH/CDC document, Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, provides thorough information. Also consult the manufacturer's operational manual.
- Monitor alarms, pressure gauges or flow indicators for any major fluctuation or changes possibly indicating a problem with the unit. DO NOT attempt to adjust the speed control or alarm settings.
- Do not disrupt the protective airflow pattern of the BSC. Make sure lab doors are closed before starting work in the BSC.
- Plan your work and proceed conscientiously.
- Minimize the storage of materials in and around the BSC.
- Hard ducted (Type B2, Total Exhaust) cabinets should be left running at all times. Cabinets that are not vented to the outside may be turned off when not in use, however, be sure to allow the BSC to run for at least 10 minutes before starting work.
- Never use fume hoods or biosafety cabinets for storage.

Before Use:

- Raise the front sash to 8 or 10 inches, as indicated on cabinet frame.
- Turn on the BSC and UV light* and let run for 5 to 10 minutes.
- Wipe down the cabinet surfaces with an appropriate disinfectant.

- Check gauges to confirm flow.
- Transfer necessary materials (pipettes, pipette tips, waste bags, etc.) into the cabinet.

*If there is an UV light incorporated within the cabinet, do not leave it on while working in the cabinet or when occupants are in the laboratory.

During Use:

- Arrange work surface from "clean" to "dirty" from left to right (or front to back).
- Keep front, side, and rear air grilles clear of research materials.
- Avoid frequent motions in and out of the cabinet as this disrupts proper airflow balance and compromises containment.

After Use:

- Leave cabinet running for at least 5 to 10 minutes after use.
- Empty cabinet of all research materials. The cabinet should never be used for storage.
- Wipe down cabinet surfaces with an appropriate disinfectant.

BSC Certification: All BSCs be tested and certified prior to initial use, relocation, after HEPA filters are changed, and at least annually. The testing and certification process includes: (1) A leak test to assure that the airflow plenums are gas tight in certain installations; (2) A HEPA filter leak test to assure that the filter, the filter frame, and filter gaskets are all properly in place and free from leaks. A properly tested HEPA filter will provide a minimum efficiency of 99.99% on particles 0.3 microns in diameter and larger; (3) Measurement of airflow to assure that velocity is uniform and unidirectional; and (4) Measurement and balance of intake and exhaust air.

Equipment must be decontaminated prior to performance of maintenance work, repair, testing, moving, changing filters, changing work programs, and after gross spills.

Training: All users must receive training prior to use of BSCs. This training is the responsibility of the PI/Faculty.

4.3.2 Centrifuge

The following requirements are designated for use of centrifuges:

- Benchtop units shall be anchored securely.
- Inspect tubes and bottles before use.
- Use only approved rotors.
- Follow all pre-run safety checks. Inspect the unit for cracks, corrosion, moisture, missing components (e.g. o-rings), etc. Report concerns immediately and tag the unit to avoid further use.
- Wipe exterior of tubes or bottles with disinfectant prior to loading.
- Operate the centrifuge per manufacturer guidelines.
 - Maintain the lid in a closed position at all times.
 - Do not open the lid until the rotor has completely stopped.
 - Do not operate the unit above designated speeds.
 - Samples are to be run balanced.
 - Do not leave the unit until full operating speed is attained and the unit is operating safely without vibration.
 - Allow the centrifuge to come to a complete stop before opening.
- Do not bump, lean on, or attempt to move the unit while it is running.
- If atypical odors or noises are observed, stop use and notify the laboratory coordinator.

- Do not operate the unit if there has been a spill.
- Inspect the unit after completion of each operation. Report concerns immediately.

4.3.3 Glassware and Test Tubes

Glassware containing biohazards should be manipulated with extreme care. Tubes and racks of tubes containing biohazards should be clearly marked with agent identification. Safety test tube trays should be used in place of conventional test tube racks to minimize spillage from broken tubes. A safety test tube tray is one that has a solid bottom and sides that are deep enough to hold all liquids if a tube should break. Glassware breakage is a major risk for puncture infections. It is most important to use non-breakable containers where possible, and carefully handle the material. Whenever possible, use flexible plastic pipettes or other alternatives. It is the responsibility of the PI and/or laboratory manager to assure that all glassware/plasticware is properly decontaminated prior to washing or disposal.

4.4 Secondary Barriers- Facility Design

4.4.1 BSL-1 Laboratory Facilities

Requirements for BSL-1 Laboratory Activities include:

- Laboratories have doors that can be locked for access control.
- Laboratories have a sink for hand washing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in the laboratory are not permitted.
- Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Laboratories with windows that open to the exterior are fitted with screens.

4.4.2 BSL-2 Laboratory Facilities

Requirements for BSL-2 Laboratory Activities (in addition to BSL-1 requirements stated above) include:

- Laboratory doors are locked when not occupied.
- 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.
- Chairs used in the laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate decontaminant. Fabric chairs are not allowed.
- An eye wash station is readily available (within 50 feet of workspace and through no more than one door).
- Ventilation - Planning of new facilities should consider ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
- 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.
- Biological Safety Cabinets (BSCs) are 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 sources of airflow disruptions.

- Vacuum lines should be protected with in-line High Efficiency Particulate Air (HEPA) filters.
- 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 either by a thimble (canopy) connection or by exhausting to the outside directly through a hard connection. Proper BSC performance and air system operation must be verified at least annually.

4.4.3 BSL-2 with BSL-3 practices Laboratory Facilities

In addition to BSL-2 and BSL-1 requirements stated above, the following is required for BSL-2 Laboratories with BSL-3 activities:

- Laboratory doors are self-closing and locked at all times. The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted.
- An entry area for donning and doffing PPE.
- The laboratory has a ducted air-exhaust system capable of directional air flow that causes air to be drawn into the work area.
- Vacuum lines are protected with in-line HEPA filters.
- All windows must be sealed.

4.4.4 BSL-3 and BSL-4 Laboratory Facilities

BSL-3 and BSL-4 activities are not anticipated for the University and therefore are not covered by this Manual. In the event these activities are anticipated, this Manual will be updated to reflect designated requirements.

4.5 Personal Protective Equipment

4.5.1 General

All laboratory personnel, regardless of and any visitors are required to abide by the following attire requirements for any entry into a University laboratory setting:

- All loose hair and clothing must be confined
- Closed-toe shoes are required
- Contact lenses are prohibited
- Entry into a laboratory where active work is performed requires the use of a lab coat and goggles, at a minimum.
- Footwear that is appropriate (minimizing slip/trip potential) for the laboratory setting shall be worn.

Additional PPE may be required as designated by the Risk Assessment. This may include: hand and face protection, respiratory protection, or the use of chemical-resistant aprons or coats.

4.5.2 Eye and Face Protection

Eye and face protection shall include the use of safety goggles or glasses at a minimum. Goggles are required for most activities and entry into active laboratories. Glasses shall be assigned for work with solid materials. For laboratory activities that involve increased splash potential, goggles/glasses shall be used in concert with face shields. The level of eye/face protection shall be assigned by the Risk Assessment.

- Entry into a laboratory setting requires the use of safety goggles, at a minimum.
- All safety glasses/goggles shall comply with the ANSI Occupational and Educational Eye and Face Protection Standard (Z87.1). Standard eyeglasses are not sufficient.
- Goggles equipped with vents to prevent fogging are recommended, and they may be worn over regular eyeglasses.
- The user shall inspect the equipment prior to each use, and clean after each use. The equipment shall fit comfortably, while maintaining adequate protection.

4.5.3 Hand Protection

Nitrile or latex gloves are required for appropriate active laboratory activity. Further protection (such as double gloving, increased chemical resistance, or different glove material) may be assigned by the Risk Assessment.

- Gloves are to be inspected prior to, and throughout use.
- Gloves are to be removed prior to leaving the laboratory using the one-hand technique. Laboratory personnel shall wash hands immediately after glove use.
- Care should be taken regarding handling of objects (pens, phones, doorknobs) that were handled while donning gloves.

4.5.4 Respiratory Protection

For activities where the Risk Assessment designates the use of Respiratory Protection, the University Respiratory Protection Program shall be implemented. This Program has been developed to meet OSHA requirements specified at 29 CFR 1910.134. These requirements include:

- Appropriate selection of respirators
- Medical pre-qualification
- Training
- Fit Testing
- Proper use, inspection and maintenance

The above elements shall be conducted through the Health and Safety Office.

4.6 Decontamination

4.6.1 Wet Heat

Wet heat is the most dependable method of sterilization. Autoclaving (saturated steam under pressure of approximately 15 psi to achieve a chamber temperature of at least 250° F for a prescribed time) rapidly achieves destruction of microorganisms, decontaminates infectious waste and sterilizes laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean it, if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation. Prevention

of entrapment of air is critical to achieving sterility. Material to be sterilized must come in contact with steam and heat.

Chemical indicators, e.g. autoclave tape, must be used with each load placed in the autoclave. The use of autoclave tape alone is not an adequate monitor of efficacy. Autoclave sterility monitoring should be conducted on a regular basis (at least monthly) using appropriate biological indicators (*B. stearothermophilus* spore strips) placed at locations throughout the autoclave. The spores, which can survive 250° F for 5 minutes but are killed at 250° F in 13 minutes, are more resistant to heat than most, thereby providing an adequate safety margin when validating decontamination procedures. Each type of container employed should be spore tested because efficacy varies with the load, fluid volume, etc.

Each individual working with biohazardous material is responsible for its proper disposition. Decontaminate all infectious materials and all contaminated equipment or labware before washing, storage or discard as infectious waste. Autoclaving is the preferred method. Never leave an autoclave in operation unattended (do not start a cycle prior to leaving for the evening). Recommended procedures for autoclaving are:

- All personnel using autoclaves must be adequately trained by their PI or lab manager. Never allow untrained personnel to operate an autoclave.
- Be sure all containment vessels can withstand the temperature and pressure of the autoclave. Be sure to use polypropylene / polyethylene autoclave bags.
- Review the operator's manual for instructions prior to operating the unit. Different makes and models have unique characteristics.
- Never exceed the maximum operating temperature and pressure of the autoclave.
- Wear the appropriate personal protective equipment (safety glasses, lab coat and heat-resistant gloves) when loading and unloading an autoclave.
- Select the appropriate cycle: liquid cycle (slow exhaust) for fluids to prevent boiling over, dry cycle (fast exhaust) for glassware, fast and dry cycle for wrapped items.
- Never place autoclave bags directly on the autoclave chamber floor. Place autoclavable bags containing waste in a secondary containment vessel to retain any leakage that might occur. The secondary containment vessel must be constructed of material that will not melt or distort during the autoclave process. (Polypropylene is a plastic capable of withstanding autoclaving but is resistant to heat transfer. Materials contained in a polypropylene pan will take longer to autoclave than the same material in a stainless steel pan.)
- Never place sealed bags or containers in the autoclave. Polypropylene bags are impermeable to steam and should not be twisted and taped shut. Secure the top of containers and bags loosely to allow steam penetration.
- Position autoclave bags with the neck of the bag taped loosely and leave space between items in the autoclave bag to allow steam penetration.
- Fill liquid containers only half full, loosen caps or use vented closures.

- For materials with a high insulating capacity (animal bedding, saturated absorbent, etc.) increase the time needed for the load to reach sterilizing temperatures.
- Never autoclave items containing solvents, volatile or corrosive chemicals.
- Always make sure that the pressure of the autoclave chamber is at zero before opening the door. Stand behind the autoclave door and slowly open it to allow the steam to gradually escape from the autoclave chamber after cycle completion.
- Allow liquid materials inside the autoclave to cool down for 15-20 minutes prior to their removal.
- Dispose of all autoclaved waste through the infectious waste stream.

4.6.2 Dry Heat

Dry heat is less efficient than wet heat and requires longer times and/or higher temperatures to achieve sterilization. It is suitable for the destruction of viable organisms on impermeable non-organic surfaces such as glass, but it is not reliable in the presence of shallow layers of organic or inorganic materials which may act as insulation. Sterilization of glassware by dry heat can usually be accomplished at 160-170° C for periods of 2-4 hours. Dry heat sterilizers should be monitored on a regular basis using appropriate biological indicators [*B. subtilis (globigii)* spore strips].

4.6.3 Incineration

Incineration is another effective means of decontamination by heat. As a disposal method incineration has the advantage of reducing the volume of the material prior to its final disposal. However, local and federal environmental regulations contain stringent requirements and permits to operate incinerators are increasingly more difficult to obtain.

4.7 Waste

All infectious waste products shall be handled in accordance with the procedures outlined in this manual in addition to the University Exposure Control Plan. All infectious waste from University laboratories must be autoclaved by the generator prior to disposal in appropriate infectious waste containers.

All biohazard waste for disposal is to be secured in each Department's waste storage area. A pickup schedule shall be established by the University's contracted vendor. Each Department Supervisor shall notify the Health and Safety Office via email if they have waste prior to each pickup. For each pickup, a University representative trained under the DOT Hazardous Materials Regulations shall review the service to confirm compliance and sign the waste manifest. All completed manifests, after receipt from the disposal facility, shall be forwarded to the Health and Safety Office for recordkeeping.

4.7.1 Categories of infectious waste

Cultures and stocks of infectious agents and associated biologicals, including the following:

- Cultures from medical and pathological laboratories;

- Cultures and stocks of infectious agents from research and industrial laboratories;
- Wastes from the production of biologicals;
- Discarded live and attenuated vaccines except for residue in emptied containers;
- Culture dishes, assemblies and devices used to conduct diagnostic tests or to transfer, inoculate and mix cultures.
- Discarded transgenic plant material.

Pathological wastes: Tissues, organs and body parts and body fluids that are removed during surgery, autopsy, other medical procedures, or laboratory procedures. Hair, nails and extracted teeth are excluded.

Human blood, blood products and body fluid waste:

- Liquid waste human blood;
- Human blood products;
- Items saturated or dripping with human blood;
- Items that are caked with dried human blood, including serum, plasma, and other blood components, which were used or intended for use in patient care, specimen testing or the development of pharmaceuticals;
- Intravenous bags that have been used for blood transfusions;
- Items, including dialysate, that have been in contact with the blood of patients undergoing hemodialysis at hospitals or independent treatment centers;
- Items contaminated by body fluids from persons during surgery, autopsy, other medical or laboratory procedures;
- Specimens of blood products or body fluids, and their containers.

Animal wastes: This category includes contaminated animal carcasses, body parts, blood, blood products, secretions, excretions and bedding of animals that were known to have been exposed to zoonotic infectious agents or non-zoonotic human pathogens during research (including research in veterinary schools and hospitals), production of biologicals or testing of pharmaceuticals. Wastes may be discarded as infectious waste or in some instances, collected by the supplier.

Isolation wastes: biological wastes and waste contaminated with blood, excretion, exudates or secretions from:

- Humans who are isolated to protect others from highly virulent diseases,
- Isolated animals known or suspected to be infected with highly virulent diseases.

Used sharps: sharps, including hypodermic needles, syringes, (with or without the attached needle), pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, culture dishes, suture needles, slides, cover slips and other broken or unbroken glass or plasticware that have been in contact with infectious agents or that have been used in animal or human patient care or treatment, at medical, research, or industrial laboratories.

4.7.2 Handling

All infectious waste from University laboratories must be autoclaved by the generator prior to disposal in appropriate infectious waste containers. The primary responsibility for identifying and disposing of infectious material rests with principal investigators or laboratory supervisors. This responsibility cannot be shifted to inexperienced or untrained personnel.

Potentially infectious and biohazardous waste must be separated from general waste at the point of generation (i.e., the point at which the material becomes a waste) by the generator into the following three classes as follows:

- Used Sharps
- Fluids (volumes greater than 20 cc)
- Other

Used sharps must be segregated into sharps containers that are non-breakable, leak proof, impervious to moisture, rigid, tightly lidded, puncture resistant, red in color and marked with the universal biohazard symbol. Sharps containers may be used until 2/3-3/4 full, at which time they must be decontaminated, preferably by autoclaving, and disposed of as infectious waste.

Fluids in volumes greater than 20 cc that are discarded as infectious waste must be segregated in containers that are leak proof, impervious to moisture, break-resistant, tightly lidded or stoppered, red in color and marked with the universal biohazard symbol. To minimize the burden of three waste categories, fluids in volumes greater than 20 cc, may be decontaminated (by autoclaving or exposure to an appropriate disinfectant), then flushed into the sanitary sewer system. The pouring of these wastes must be accompanied by large amounts of water. The empty fluid container may be autoclaved, then discarded with other infectious waste if it is disposable or autoclaved and washed if reusable.

Other infectious waste must be discarded directly into containers or plastic (polypropylene) autoclave bags that are clearly identifiable and distinguishable from general waste. Containers must be marked with the universal biohazard symbol. Autoclave bags must be distinctly colored red or orange, and marked with the universal biohazard symbol. These bags must not be used for any other materials or purpose.

Infectious waste that is decontaminated on the same floor or within the same building must be carried in a closed, durable, non-breakable container labeled with the biohazard symbol. Materials transported to other facilities must be packaged in a closed durable, non-breakable container labeled with the biohazard symbol.

4.7.3 Storage

Infectious waste must not be allowed to accumulate. Contaminated material should be inactivated and disposed of daily or on a regular basis as required. If the storage of contaminated material is necessary, it must be done in a rigid container away from general traffic and labeled appropriately.

Infectious waste, excluding used sharps, may be stored at room temperature until the storage container is full, but no longer than 30 days from the date of generation. It may be refrigerated for up to 30 days and frozen for up to 90 days from the date of generation. Infectious waste must be dated when refrigerated or frozen for storage.

4.7.4 Monitoring Treatment of Infectious Waste

Autoclaving of infectious waste must be monitored to assure the efficacy of the treatment method. A log noting the date, test conditions and the results of each test of the autoclave must be kept.

Section 5: Animal Safety

There are four animal biosafety levels (ABSLs), designated as ABSL-1, ABSL-2, ABSL-3 and ABSL-4, for work with infectious agents in mammals. The levels are combinations of practices, safety equipment and facilities for experiments on animals infected with agents that produce or may produce human infection. In general, the biosafety level recommended for working with an infectious agent in vivo and in vitro is comparable.

ABSL-1 is suitable for work involving well characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.

ABSL-2 is suitable for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-3 is suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease.

ABSL-4 is suitable for addressing dangerous and exotic agents that pose high risk of like threatening disease, aerosol transmission, or related agents with unknown risk of transmission.

A summary of Containment and Control measures is provided in Table 3, below. Complete descriptions of all Biosafety Levels and Animal Biosafety Levels are outlined in the 5th edition of Biosafety in Microbiological and Biomedical Laboratories published by the U. S. Department of Health and Human Services (CDC/NIH).

Table 3			
Recommended Animal Biosafety Levels (ABSL)			
ABSL	Laboratory Practices	Primary Barriers (Safety Equipment)	Secondary Barriers (Facility Design)
1	Standard animal care and management practices, including medical surveillance	As required for normal care of each species.	* Restricted access as appropriate * No recirculation of exhaust air * Recommend directional air flow * Hygiene facilities recommended
2	ABSL-1 practices plus: * Biohazard warning signs * Sharps precautions * Decontamination * Dedicated SOP	ABSL-1 equipment plus: * Animal containment equipment appropriate for animal species PPE: laboratory coats; gloves; face and respiratory protection as needed	ABSL-1 facility plus: * Limited access * Autoclave available * Hygiene facilities * Mechanical cage washer used
3	ABSL-2 practices plus: * Decontamination of clothing before laundering * Cages decontaminated before bedding removed * Disinfectant foot bath as needed	ABSL-2 equipment plus: * Containment equipment for housing animals and cage dumping activities * Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPE: laboratory coats; gloves; face and respiratory protection as needed	ABSL-2 facility plus: * Controlled access * Physical separation from access corridors * Self-closing, double-door access * Sealed penetrations and windows * Autoclave available in facility

4	ABSL-3 practices plus: * Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting * All wastes are decontaminated before removal from facility	ABSL-3 equipment plus: * Maximum containment equipment (e.g. Class III BSCs or partial containment equipment in combination with full-body, air-supplied, positive pressure personnel suit) used for all procedures and activities	ABSL-3 facility plus: * Separate building or isolated zone * Dedicated supply and exhaust, vacuum, and decon systems * Specific design requirements outlined by CDC
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**There are currently no activities permitted by The University under the scope of this manual that involve ABSL-4 agents.*

Section 6: Emergencies

6.1 Emergencies

Emergencies involving biohazards are outlined in this section. For emergencies involving chemical hazards, refer to the University Chemical Hygiene Plan.

6.2 Reporting

All incidents, exposures, spills, etc. are to be immediately reported to the faculty member/Principal Investigator. The controlling faculty member/Principal Investigator is responsible for completing the Accident Report form found in Appendix B and forwarding it to the Health and Safety Office.

OSHA Recordkeeping: An exposure incident is evaluated to determine if the case meets OSHA's Recordkeeping Requirements (29 CFR 1904), where not exempt. This determination and the recording activities shall be completed by the Human Resources office.

Sharps Injury Log: In addition to the 1904 Recordkeeping Requirements, all percutaneous injuries from contaminated sharps are also recorded in a Sharps Injury Log. All incidences must include at least:

- Date of the injury
- Type and brand of the device involved (syringe, suture needle)
- Department or work area where the incident occurred
- An explanation of how the incident occurred.

This log is reviewed as part of the annual program evaluation and maintained for at least five years following the end of the calendar year covered. If a copy is requested by anyone, it must have any personal identifiers removed from the report. The Sharps injury log is maintained by the Human Resources office.

6.3 Biological Spill Procedures

Spills must be cleaned up as soon as practical in accordance with the following protocol. Employees must be trained in accordance with this plan and applicable spill procedures prior to attempting remediation. Spill kits shall be readily available in each laboratory and contain disinfectants; absorbing materials (pads, towels, etc.); PPE; mechanical device for removing sharps (forceps, tongs, scoops, pans); and, disposal container.

For Emergencies within a BSL-1 Laboratory and blood-related cleanup incidents:

1. Evacuate- Remove individuals from the immediate work area (e.g. the room where the spill occurred).
2. Notify- Notify the faculty member/Principal Investigator and, if necessary, the Health and Safety Office.
3. PPE- Don PPE, including at a minimum: lab coat, goggles and gloves. Additional PPE may include a face shield and body/sleeve protection.
4. Sharps- Remove any broken glass or other large materials and immediately containerize. Use forceps or similar device to avoid injury.
5. Gross Cleanup- Remove gross material through the use of absorbent material.
6. Apply the assigned disinfectant solution (or 10% bleach solution) to the area. Work from the outer limits of the spill towards the center. Ensure adequate contact time is obtained as directed by the product manufacturer (or 20-30 minutes for bleach).
7. After contact time is obtained, again wipe the area with heavy towels from outside-in.
8. All non-sharp material shall be placed into a red biohazard bag. Sharps shall be placed into an assigned sharps container.
9. Remove goggles/face shield, body coverings, and then gloves. Immediately wash hands with soap and water.

For Emergencies within a BSL-2 Laboratory

1. Evacuate- Remove individuals from the immediate work area (e.g. the room where the spill occurred). Close lab door and post Do Not Enter or place caution tape across door.
2. Notify- Notify the faculty member/Principal Investigator and, if necessary, the Health and Safety Office.
3. In the event of an exposure, remove contaminated clothing and wash exposed skin with soap and water.
4. PPE- Don PPE, including at a minimum: lab coat, goggles and gloves. Additional PPE may include a face shield and body/sleeve protection.
5. Allow aerosols to settle for at least 30 minutes before re-entering the area.
6. Sharps- Remove any broken glass or other large materials and immediately containerize. Use forceps or similar device to avoid injury.
7. Gross Cleanup- Remove gross material through the use of absorbent material.
8. Apply the assigned disinfectant solution (or 10% bleach solution) to the area. Work from the outer limits of the spill towards the center. Ensure adequate contact time is obtained as directed by the product manufacturer (or 20-30 minutes for bleach).
9. After contact time is obtained, again wipe the area with heavy towels from outside-in.
10. All non-sharp material shall be placed into a red biohazard bag. Sharps shall be placed into an assigned sharps container.
11. Remove goggles/face shield, body coverings, and then gloves. Immediately wash hands with soap and water.

**There are currently no activities permitted by The University under the scope of this manual that involve BSL-3 or BSL-4 agents. In the event this Manual is modified to cover these agents, emergency actions within these laboratories shall be developed during the Risk Assessment phase outlined in Section 3 of this Plan.*

6.4 Incident Review

The faculty member/Principal Investigator and the IBC will review the circumstances of all incidents to determine:

- Engineering controls in use at the time
- Work practices followed
- Protective equipment or clothing that was used at the time of the incident (gloves, eye shields, etc.)
- Location of the incident
- Procedure being performed when the incident occurred
- Personnel training

If revisions to this plan are necessary, the IBC and Health and Safety Office will ensure that appropriate changes are made. Changes may include an evaluation of the Risk Assessment, safer devices, additional training, etc.



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Appendix A

**ABSA/OSHA Alliance Program:
Principles of Good Microbiological Practice**

**University of Scranton
Biosafety Plan
May 2014**



PRINCIPLES OF GOOD MICROBIOLOGICAL PRACTICE

1. Never use mouth pipette. Avoid hand to mouth or hand to eye contact in the laboratory. Never eat, drink, apply cosmetics or lip balm, handle contact lenses or take medication in the laboratory.
2. Use aseptic techniques. Hand washing is essential after removing gloves and other personnel protective equipment, after handling potentially infectious agents or materials and prior to exiting the laboratory.
3. CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) recommends that laboratory workers protect their street clothing from contamination by wearing appropriate garments (eg, gloves and shoe covers or lab shoes) when working in *Biosafety Level-2 (BSL-2)* laboratories. In *BSL-3* laboratories the use of street clothing and street shoes is discouraged; a change of clothes and shoe covers or shoes dedicated for use in the lab is preferred. *BSL-4* requires changing from street clothes/shoes to approved laboratory garments and footwear.
4. When utilizing sharps in the laboratory, workers must follow OSHA's Bloodborne Pathogens standard requirements. Needles and syringes or other sharp instruments should be restricted in laboratories where infectious agents are handled. If you must utilize sharps, consider using safety sharp devices or plastic rather than glassware. Never recap a used needle. Dispose of syringe-needle assemblies in properly labeled, puncture resistant, autoclavable sharps containers.
5. Handle infectious materials as determined by a risk assessment. Airborne transmissible infectious agents should be handled in a certified Biosafety Cabinet (BSC) appropriate to the biosafety level (BSL) and risks for that specific agent.
6. Ensure engineering controls (e.g., BSC's, eyewash units, sinks, and safety showers) are functional and properly maintained and inspected.
7. Never leave materials or contaminated labware open to the environment outside the BSC. Store all biohazardous materials securely in clearly labeled, sealed containers. Storage units, incubators, freezers or refrigerators should be labeled with the *Universal Biohazard* sign when they house infectious material.
8. Doors of all laboratories handling infectious agents and materials must be posted with the Universal Biohazard symbol, a list of the infectious agent(s) in use, entry requirements (e.g., PPE) and emergency contact information.
9. Avoid the use of aerosol-generating procedures when working with infectious materials. Needle clipping, pipetting mixing, sonication, and centrifugation can produce substantial aerosols. If you must perform an aerosol generating procedure, utilize proper containment devices and good work practice controls to mitigate potential exposures; Tightly cap tubes prior to centrifuging or vortexing; Allow aerosols to settle prior to opening tubes, equipment; Open tubes or equipment inside a containment device whenever feasible; Shield instruments or activities that can emit splash or splatter.
10. Use disinfectant traps and in-line filters on vacuum lines to protect vacuum lines from potential contamination.
11. Follow the laboratory biosafety plan for the infectious materials you are working with and use the most suitable decontamination methods for decontaminating the infectious agents you use. Know the laboratory plan for managing an accidental spill of pathogenic materials. Always keep an appropriate spill kit available in the lab.
12. Clean laboratory work surfaces with an approved disinfectant after working with infectious materials. The containment laboratory must not be cluttered in order to permit proper floor and work area disinfection.
13. Never allow contaminated, infectious waste materials to leave the laboratory or to be put in the sanitary sewer without being decontaminated or sterilized. When autoclaving use adequate temperature (121°C), pressure (15 psi), and time, based on the size of the load. Also use a sterile indicator strip to verify sterilization. Arrange all materials being sterilized, so as not to restrict steam penetration.
14. When shipping or moving infectious materials to another laboratory, always use U.S. Postal or Department of Transportation (DOT) approved, leak-proof sealed and properly packed containers (primary and secondary containers). Avoid contaminating the outside of the container and be sure the lid is on tight. Decontaminate the outside of the container before transporting. Ship infectious materials in accordance with Federal and local requirements.
15. Report all accidents, occurrences and unexplained illnesses to your work supervisor and the Occupational Health Physician. Understand the pathogenesis of the infectious agents you work with.
16. Think safety at all times during laboratory operations. Remember, if you do not understand the proper handling and safety procedures or how to use safety equipment properly, do not work with the infectious agents or materials until you get instruction. Seek the advice of the appropriate individuals. *Consult the CDC/NIH BMBL for additional information. Remember, following these principles of good microbiological practices will help protect you, your fellow worker and the public from the infectious agents you use.*

Through OSHA's Alliance Program, this Fact Sheet was developed as a product of the OSHA and American Biological Safety Association Alliance for informational purposes only. It does not necessarily reflect the official views of OSHA or the U.S. Department of Labor.



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Appendix B

IBC New Investigator Registration Sheet

University of Scranton

Biosafety Plan

May 2014



INSTITUTIONAL BIOSAFETY COMMITTEE

University of Scranton New Investigator Registration Sheet

Overview: The University of Scranton Institutional Biosafety Committee will review this document and contact you regarding specific forms, if any, that will be required for institutional approval of your work.

Name of Principal Investigator: _____ Department: _____

Title of Project: _____

Proposed Start Date of Project: _____ Expected Duration of Project: _____

The proposed work will involve the following:

- YES NO Recombinant DNA
- YES NO Transgenic Organisms
- YES NO Human Body Fluids, Tissues and/or Cell lines
- YES NO Plant or animal pathogens, toxins, federally regulated agents and toxins, viral vectors
- YES NO Radioisotopes (If YES, Radiation Safety Committee approval required.)
- YES NO Animal Subjects (If YES, IACUC approval is required.)
- YES NO Human Subjects (If YES, IRB approval is required.)

- Attach a brief description of your procedure(s) (two pages maximum including information pertaining to any topics checked yes above). If using human materials, attach MSDS documentation.
- Attach a description of the procedures you will use to dispose of human materials or decontaminate biohazardous materials.
- Attach a list of personnel and any training and/or personal protective equipment needed for those involved with the proposed project.

Signature _____

Date _____

Return to: Institutional Biosafety Committee
Office of Research and Sponsored Programs
IMBM, Rm203, University of Scranton

(rev. 9/10)



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Appendix C

Biological Agents and Associated BSL/Risk Group

University of Scranton

Biosafety Plan

May 2014



Biosafety Plan

Appendix C: References for Biological Agents and Associated BSL/Risk Group

Source: American Biological Safety Association: Risk Group Classification for Infectious Agents

<http://www.absa.org/riskgroups/index.html>



Risk Group Classification for Infectious Agents

In many countries, including the United States, infectious agents are categorized in risk groups based on their relative risk. Depending on the country and/or organization, this classification system might take the following factors into consideration:

- Pathogenicity of the organism
- Mode of transmission and host range
- Availability of effective preventive measures (e.g., vaccines)
- Availability of effective treatment (e.g., antibiotics)
- Other factors

Risk Group Database

[Search Bacteria](#) [Search Viruses](#) [Search Fungi](#) [Search Parasites](#)

[Leave feedback about this database \(questions, comments or suggestions\) >](#)

Bacteria Search

Genus Species

Human Pathogen Animal Pathogen Plant Pathogen

Select Agent CDC Select Agent USDA

NOTE: Large searches take a few seconds to load

..or choose genus from list below

Abiotrophia, Acetivibrio, Achleplasma, Achromobacter, Acidaminococcus, Acidovorax, Acinetobacter, Actinobacillus, Actinobaculum, Actinomadura, Actinomyces, Aegyptianella, Aerococcus, Aeromonas, Afipia, Alcaligenes, Alloiooccus, Allomonas, Alteromonas, Amycolata, Anaerobiospirillum, Anaerorhabdus, Anaplasma, Arachnia, Arcanobacterium, Arcobacter, Arizona, Arsenophonus, Arthrobacter, Atopobium, ba, Bacillus, Bacteroides, Balnearix, Bartonella, Beneckea, Bergeyella, Bifidobacterium, Bilophila, Bordetella, Borrelia, Brachyspira, Brevibacterium, Brevinema, Brevundimonas, Brucella, Burkholderia, Calymmatobacterium, Campylobacter, Capnocytophaga, Cardiobacterium, Carnobacterium, Catonella, Cedeccea, Cellulomonas, Centipeda, Chlamydia, Chlamydomphila, Chromobacterium, Chryseobacterium, Citrobacter, Clavibacter, Clostridium, Comamonas, Corynebacterium, Cowdria, Coxiella, Curtobacterium, Cytophaga, Dermatophilus, Dermatophilus, Dialister, Dichelobacter, Dolosigranulum, Edwardsiella, Ehrlichia, Eikenella, Empedobacter, Enterobacter, Enterococcus, Eperythrozoon, Erlichia, Erwinia, Erysipelothrix, Escherichia, Eubacterium, Eurbacterium, Ewingella, Facklamia, Faenia, Falcivibrio, Flavobacterium, Flexibacter, Fluoribacter, Francisella, Fusobacterium, Gardnerella, Gemella, Globicatella, Gordonia, Haemobartonella, Haemophilus, Hafnia, Hallella, Hartmanella, Helcococcus, Helicobacter, Herellea, Johnsonella, Jonesia, Kingella, Klebsiella, Kluyvera, Koserella, Lactobacillus, Lactococcus, Lawsonia, Leclercia, Legionella, Leptospira, Levinea, Liberobacter, Listeria, Listonella, Mannheimia, Megasphaera, Melissooccus, Microvirgula, Mima, Mitsukella, Mobiluncus, Moraxella, Moraxella (Branhamella), Morganella, Morococcus, Mycobacterium, Mycoplasma, Myroides, Neisseria, Neorickettsia, neurolyticum, Nocardia, Nocardioopsis, Nocardia, Ochrobactrum, Oligella, Orienta, Ornithobacterium, Paenibacillus, Pantoea, Pasteurella, Peptococcus, Peptostreptococcus, Photobacterium, Piscirickettsia, Plesiomonas, Porphyromonas, Prevotella, Propionibacterium, Proteus, Providencia, Pseudalteromonas, Pseudomonas, Pseudoramibacter, Psychrobacter, Ralstonia, Renibacterium, Rhodococcus, Rickettsia, Riemerella, Rochalimaea, Saccharopolyspora, Salmonella, Sanguibacter, Selenomonas, Serpulina, Serratia, Serretia, Shewanella, Shigella, Sphaerophorus, Sphingobacterium, Sphingomonas, Spiroplasma, Sporichthya, Staphylococcus, Stenotrophomonas, Streptobacillus, Streptococcus, Streptomyces, Sutterella, Suttonella, Tatlockia, Tatumella, Taylorella, Tissierella, Treponema, Tsukamurella, Turicella, Ureaplasma, Vagococcus, Veillonella, Vibrio, Waddlia, Xanthomonas, Xylella, Xylophilus, Yersinia

Viruses Search

Viral Group Name

Human Pathogen Animal Pathogen

Select Agent CDC Select Agent USDA

NOTE: Large searches take a few seconds to load

..or choose viral group from list below

Adenoviridae, Arenaviridae, Astroviridae, Baculoviridae, Birnaviridae, Bornaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Filoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Iridoviridae, Orthomyxoviridae, Papovaviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Poxviridae, Prion, Reoviridae, Retroviridae, Rhabdoviridae, Togaviridae, Toroviridae, Unclassified



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Appendix D

Incident Report Form

University of Scranton

Biosafety Plan

May 2014

BIOSAFETY INCIDENT REPORT

Date of Incident	Time	Incident Location
Protocol Number	Principal Investigator/Faculty Member	
List all persons present		
Describe what happened		
Signature of person submitting report		Date
Signature of Principal Investigator		Date

To be completed by the Institutional Biosafety Committee (IBC):

Incident reviewed by	Date
Findings	
Recommendations	