CASE WESTERN RESERVE UNIVERSITY
Department of Environmental Health and Safety

Biosafety Manual

(revised 2025)

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Introduction

Biosafety Manual Overview

This Biosafety Manual compiles information from various universities and government agencies to protect workers and the environment while ensuring compliance with relevant standards and regulations. Although it does not cover every aspect of biosafety, it outlines essential procedures, safety precautions, and general rules critical for laboratory work involving biological agents.

All laboratory personnel must adhere to safe work practices and follow established safety guidelines. Understanding the risks associated with pathogens and biohazardous materials is vital for maintaining a safe working environment.

Laboratory staff are required to familiarize themselves with the CDC's *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, as these resources must be thoroughly understood before engaging in any biological work. Knowledge of general laboratory standard practices is also necessary.

Safety is the top priority for all experiments. Planning and implementing biohazard controls to prevent laboratory-associated infections and manage contamination must be integral to all activities involving biohazardous agents, including recombinant or synthetic DNA/RNA. This manual aims to ensure that university laboratories using biological agents adhere to these procedures and guidelines. For questions about the categorization, handling, storage, treatment, or disposal of biohazardous materials, laboratory workers should contact the University Biosafety Officer in Environmental Health and Safety.

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Chapter 1 – Management Structure and Responsibility

Commitment to Safety

It is the objective of all Case Western Reserve University laboratories, and their management, to practice safety in science and to exercise all reasonable and prudent precautions generally accepted as research industry standards. Guidelines recommended by the CDC and NIH for biosafety at levels 1 - 4 will be strictly observed and enforced by the PI and assigned laboratory facility management. At the conclusion of initial safety training by EHS, each laboratory employee will be trained by the CHO and the ECO responsible for that laboratory.

Dual Use Research of Concern (DURC)

Certain types of life sciences research may have the potential to be misapplied for harmful purposes, referred to as Dual Use Research of Concern (DURC). While DURC is not common in most routine laboratory operations, the institution is committed to identifying and managing DURC in compliance with applicable federal policies.

The University will evaluate proposed research to determine if it involves any of the 15 agents or toxins and 7 categories of experiments specified in the U.S. Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (September 2014). Research that potentially qualifies as DURC will be subject to additional institutional oversight, risk mitigation planning, and federal notification as required.

Principal Investigators must disclose any research that may involve DURC agents or experimental categories during the IBC protocol review process. The IBC, in consultation with Environmental Health and Safety and institutional leadership, will determine whether the research constitutes DURC and what additional oversight is warranted.

Laboratory Management Responsibilities

Overall supervision and management of the laboratory is the responsibility of the PI or their designee. Access to the laboratory must be obtained from this individual.

It must be noted that the University cannot create an all-inclusive document that will meet the conditions and needs of each of the 300+ laboratories on the campus. Application of the University’s policies is the responsibility of each laboratory's ECO. The CHO, in most cases, will serve as the ECO and will be responsible for the overall management and support of the laboratory's Bloodborne Pathogen Compliance Program. In all cases, the Primary Investigator is the final authority in the laboratory. Some of the specific duties of this individual are:

1. Exposure determination (identification of affected employees)
2. Creation and modification of the site specific ECP
3. Annual site-specific retraining
4. Assurance that employees receive the appropriate OSHA and University required safety training and annual retraining from EHS
5. Assurance that the principles of the ECP are followed in the laboratory
6. Collection of information and notification to both University Health Services and EHS if an exposure incident occurs

Laboratory Access

Access to the laboratory is authorized by the PI or their designee. Anyone requesting to use the lab or its equipment must be informed of potential hazards and the biosafety guidelines outlined in this manual.

Access is restricted during work with infectious agents, after hours, or when personnel are unavailable. Individuals at increased risk for infection, such as minors and those who are immunosuppressed or undergoing immunosuppressive therapy, are not permitted in the laboratory.

Laboratory Security

Certain biohazardous microorganisms and toxins can pose significant risks if not handled properly. Therefore, infectious agents must be secured within the laboratory.

The highest level of security is reserved for materials classified as Select Agents. Working with these agents requires a Biosecurity Plan and authorization from the University. Currently, no work with Select Agents is conducted at Case Western Reserve University.

If a request is received from another institution or corporate entity for a hazardous organism for academic purposes, the PI is responsible for verifying that the receiving entity is a legitimate research organization and that the transfer has administrative approval from both institutions. Upon receiving such a request, the PI must notify the University's Biological Safety Officer for approval to send or receive any agent.

Chemical Hygiene Plan (CHP)

All laboratories working with chemicals need a CHP and need to follow the requirements prescribed in *29 CFR part 1910.1450* "Occupational exposure to hazardous chemicals in laboratories". Most laboratories working with bloodborne pathogens also use chemicals, and therefore need a CHP, as well as an ECP. The Laboratory Safety Standard requires employers to write and implement laboratory-specific CHP. The CHP must accompany the *Case Western Reserve University Laboratory Safety Manual*. According to this regulation, a CHP applies to all employers engaged in the laboratory use of hazardous chemicals. A "laboratory" is defined as a facility where hazardous chemicals are used, and a "hazardous chemical" is one that poses acute or chronic health risks to employees. The CHP should outline work practices and policies to protect employees from these chemicals. The CHP should include specific work practices, procedures, and policies to ensure that employees are protected from all potentially hazardous chemicals used. Each lab must appoint a Chemical Hygiene Officer (CHO), usually the Principal Investigator (PI), to develop, review, and train staff on the CHP.

Hard copies of the CHP, including updates, must be submitted to Environmental Health and Safety (EHS). A customizable CHP template is available on the EHS website.

Exposure Control Plan (ECP) – Bloodborne Pathogen Standard

The OSHA standard involving **bloodborne pathogens** can be divided into several broad categories that are part of a written document known as the ECP, which is prescribed in *29 CFR part 1910.1030 (c)(1)(i)*. These include:

1. Exposure Control Officer (ECO) will in most cases be the PI of the laboratory. In cases where the ECO is not the PI, The PI will retain final authority in the laboratory but will allow the designated ECO to perform day to day operations in their absence. The name of the Exposure Officer and the name of the PI must be included in the ECP, along with their telephone numbers. The plan must also describe the location of the laboratory where this plan is to be used. All employers having employees with occupational exposure are required to establish a written ECP addressing the OSHA standard and notify affected employees of the plan and its purpose.

2. Exposure Determination Employers must evaluate all personnel for occupational exposure to bloodborne pathogens, defined as "any reasonably anticipated skin, eye, mucous membrane or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties." In order to determine which employees require training, employers must list all employee positions according to the following categories:

    a. CATEGORY I - A list of job classifications in which employees in those job classifications will **ALWAYS** have occupational exposure.

    b. CATEGORY II - A list of job classifications in which employees will **SOMETIMES** have occupational exposure.

c. CATEGORY III – A list of job classifications in which employees will **NEVER** have occupational exposure.

3. Employee List and Categories: The ECP must include a list of all laboratory employees and their exposure categories. Those in Categories I and II must undergo annual bloodborne pathogen training. This list should be updated whenever there are personnel changes, such as terminations, new hires, or duty changes.
4. Universal Precautions: Adopted by OSHA and recommended by the CDC, universal precautions treat all human blood and certain body fluids as potentially infectious. Key components include:

    - **Barrier Precautions:** Use of gloves, masks, protective eyewear, and gowns.
    - **Handwashing:** Regular hand hygiene practices.
    - **Sharps Precautions:** Safe disposal of needles and other sharps in designated containers.

At CWRU, specific practices are enforced:

- Items in red or orange containers/bags are treated as potentially infectious.
- All needles, syringes, and blades are considered hazardous and must be disposed of in red rigid sharps containers, as per Ohio law.
- Unlabeled or unidentified materials are treated as bloodborne pathogens for disposal.

The ECO is responsible for ensuring all employees understand and follow these universal precautions.

5. Hazard Control Methods must be utilized to protect the workers, the public and the environment from exposure to dangerous materials, in this case, biohazards. These methods include:

    a. Engineering Controls, which reduce or eliminate hazards at the source and include:

    Primary Barriers - Containment of aerosol-generating biohazardous materials that pose an inhalation risk. Biological safety cabinets and their respective ventilation systems are the preferred method for the primary containment of biohazards.

    i. A Biosafety Cabinet is designed to contain microorganisms, which are released during work within the cabinet.

    The Class I biosafety cabinet is an open-front negative pressure cabinet, which does not have to be vented, making it suitable for use in laboratory rooms which cannot be ducted. The exhaust air from the cabinet is filtered by

a high-efficiency particulate air (HEPA) filter, which recirculates clean air back into the room. This cabinet is acceptable for use of low to moderate risk agents in the absence of volatile toxic chemicals and volatile radionuclides. The Class I biosafety cabinet will provide personnel and environmental protection, but not product protection.

The Class II vertical laminar-flow biological cabinet is an open-front, ventilated cabinet, which provides HEPA-filtered, recirculated mass airflow within the workspace, as well as HEPA-filtered exhaust air from the cabinet through a duct system. Thus, the Class II biosafety cabinet will provide personnel, environment and product protection. While HEPA filters are effective for trapping particulates and infectious agents, these filters will not capture volatile chemicals or gases. There are four types of Class II biosafety cabinets.

Class II, type A: This does not have to be vented, which makes it suitable for use in laboratory rooms which cannot be ducted. It is vented, with 30% of the air exhausted outside and 70% is recirculated back into the cabinet. No toxic or flammable material should be used in these types of cabinets due to the possibility of rapid increase in concentration

Class II, type B1: This cabinet is vented, with 70% of the air exhausted outside the laboratory and 30% is recirculated back into the room. This cabinet may be used with etiologic agents treated with minute quantities of toxic chemicals and trace amounts of radionuclides required as an adjunct to microbiological studies if work is done in the directly exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the downflow air.

Class II, type B2: This cabinet must be totally exhausted, with 100% of the air exhausted through a dedicated ducting system. This cabinet may be used with etiologic agents treated with toxic chemicals and radionuclides required as an adjunct to microbiological studies.

Class II, type B3: This cabinet has been replaced with type A, but many B3 cabinets still remain in use on campus. It is vented, with 30% of the air exhausted outside and 70% is recirculated back into the cabinet. No toxic or flammable material should be used in these types of cabinets due to the possibility of rapid increase in concentration

Refer to the BMBL Primary Containment for Biohazards, for selection, installation and use of Biological Safety cabinets. Three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories are described in Appendix A of the BMBL. Open-fronted Class I and Class II biological safety cabinets

are primary barriers which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

Another example of a primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize this hazard, containment controls such as BSCs or centrifuge cups must be used for handling infectious agents that can be transmitted through the aerosol route of exposure. Safety equipment also may include items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles.

PPE is often used in combination with biological safety cabinets and other devices which contain the agents, animals, or materials being worked with. In some situations, in which it is impractical to work in biological safety cabinets, PPE may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

Horizontal laminar flow "clean benches" are used in clinical, pharmaceutical, and laboratory facilities strictly for *product* protection. This equipment **MUST NEVER** be used when handling toxic, infectious, radioactive, or sensitizing materials, since the worker sits in the immediate downstream exhaust from the "clean bench." Vertical laminar flow benches may be useful for certain manipulations of clean materials (e.g., pouring agar plates) but should not be used when working with infectious materials.

ii. Secondary Barriers (e.g., building design features include floor to ceiling walls, operating areas under negative pressure and use of closed doors). Many laboratories have built-in monitoring systems to indicate any system failures that could affect secondary containment. Laboratory personnel should be familiar with these devices if they are available.

iii. Sharps Handling – This includes equipment and devices (e.g., sharps disposal containers, self-sheathing needles, safer medical devices, such as sharps with engineered sharps injury protections and needleless systems) that isolate or remove the bloodborne pathogens hazard from the workplace.

b. Work Practice applications are administrative rules that control hazards between the worker and the hazard source. Example: Sweeping up broken glass instead of picking it up with your hands. It also includes the reduction of a worker's exposure through the management of time spent with, and/or distance spent from the hazard source. Universal precautions and University policy further describe two work practice controls:

   i. Hand washing - Hands and other skin surfaces must be washed as soon as feasible if contaminated with blood or body fluids. The use of gloves does not preclude the necessity for hand washing. When hand washing facilities are not available, antiseptic hand cleaners or towelettes must be provided.

   ii. SHARPS Precautions - SHARPS designed for use in biological, etiological, bacteriological or tissue culture work are defined as: discarded hypodermic needles, syringes, and scalpel blades, cannulas, coverslips, microscope slides, all pipettes (glass or plastic) and pipette tips, test tubes, broken Petri dishes, broken glass or any other item capable of causing puncture wounds or cuts.

   All hypodermic needles, syringes, scalpel blades, razor blades, cannulas, coverslips, broken glass pipettes, and microscope slides must be placed in puncture-resistant, leakproof containers that are labeled or color-coded in accordance with this standard. These containers must have a closable cover and be closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

   These containers must also be easily accessible to personnel, maintained upright throughout use and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found. They must be replaced routinely and not be allowed to overfill. When moving containers of contaminated sharps from the area of use, the containers must be placed in a second closable container that is also leakproof. Hypodermic needles should not be recapped, clipped, broken or disassembled prior to disposal.

c. Personal Protective Equipment (PPE) includes all apparel that shields the employee from exposure while working with hazardous substances or biohazardous materials. Before the required PPE is donned, it is important to be wearing appropriate attire for work in a laboratory. This should include:

   i. Normal apparel should extend below the lab coat. Full-length trousers are strongly encouraged.

   ii. Sleeved shirts. Sleeveless shirts and tank tops should not be worn while conducting laboratory procedures of any kind.

iii. Proper footwear. Open-toed shoes and sandals are forbidden when working in laboratories, especially when working with hazardous chemicals, glassware or sharps.

PPE should be selected in accordance with the identified hazards associated with laboratory hazard assessment. All laboratory personnel must wear appropriate PPE corresponding to the chemical, biological and radiological substances being used. All items of PPE must be removed and properly disposed of when leaving the work area. PPE must only be worn in laboratory work areas and MUST NEVER be worn in general public areas. Appropriate PPE include:

i. Proper gloves – Latex gloves can be used when working with biological agents. Other types of gloves, such as nitrile, can be used in place of latex, and use of such gloves is increasingly encouraged because of some allergenic effects of latex. When simultaneously working with chemicals, the choice of glove material becomes most important. There is not one glove material that protects against all chemicals. To determine what type of glove the laboratory personnel should be wearing, refer to a glove selection chart. Glove selection charts are available through various laboratory supply catalogs and at the EHS website. Gloves must be worn when direct contact with blood or other potentially infectious body fluids is expected to occur, when examining abraded or non-intact skin, during invasive procedures, when the employee has cuts, lesions, dermatitis, or chapped hands, and in situations involving phlebotomy. Some procedures, where bloodborne pathogens are encountered, may call for the use of gloves containing material such as metal mesh (autopsies) or Kevlar (law enforcement, emergency and rescue crews). In this case, one or two layers of the proper disposable latex or polymeric glove should be worn over these more expensive protective gloves.

   There are three modes of glove failure:

   - **Permeation** - The ability of substances to leach through the glove's material over time. For this reason, it is important to be cognizant of chosen glove limitations using the manufacturer's glove chart.
   - **Degradation -** All polymeric gloves eventually deteriorate, as natural atmospheric elements such as ozone, UV and free radicals break down the polymer structure of the material, causing it to become brittle. Gloves should be periodically checked and disposed of when they have reached the end of the manufacturer's suggested shelf life.
   - **Penetration -** A glove's inability to withstand tears or punctures from sharp objects such as needles, scalpels, razor blades or broken glass. This limits a glove's useful lifetime. For this reason, extra care should be taken when working with contaminated sharps.

   Further regulations governing the use of gloves state that:

- Gloves must be of appropriate size, material and quality.
- Gloves must be available that are hypoallergenic or otherwise designed to minimize allergic reactions.
- Gloves should be immediately changed when damaged or contaminated.

ii. Appropriate eye protection – Safety glasses and face shields have no seal around the eyes and therefore ONLY protect them from solid particles directly in front of the worker. Safety goggles, which protect the eyes from both solids and liquids, are required when working with both hazardous liquid chemicals and biohazardous liquids.

iii. Proper body protection – LABORATORY COATS (mandatory at the University), scrubs, specific-hazard resistant gowns or aprons, protective sleeves, suits, hoods and foot covers are all components of approved protective body wear. Gowns, aprons or other protective clothing must be worn when contamination from aerosolization or spattering of blood or other body fluids is anticipated. Gowns and aprons must be appropriate for the procedure involved. The type and characteristics of this body protection depends upon the task and degree of exposure that might occur.

Gowns and other protective clothing must not permit blood or body fluids to pass through and reach undergarments, skin, mouth, eyes, etc. under normal conditions of use.

iv. Respirator protection – There are two types of respirators, air purifying and air supplied.

- **Air purifying respirators** can be particulate filtering, as found in the N95 mask-type, or those utilizing chemical absorptive cartridges found on half and full face respirators and powered air purifying respirators (PAPR). Air purifying respirators do not provide protection in oxygen deficient areas or where dangerous temperatures exist. It is important to note that surgical masks ARE NOT respirators.
- **Air Supplied respirators** operate on air fed from a non-hazardous source. The source of air may come from air tanks, or an air pump placed in an area where fresh air is available. An air supplied respirator is the only type of respirator that can be used in oxygen deficient zones.

All individuals requiring respirators need a medical evaluation by Health Services, respirator training and a fit test by EHS. These three requirements must be conducted annually to permit continuing respirator usage.

The minimum level of protective equipment in all University laboratories include lab coats, proper eye protection and appropriate gloves. Lab coats

MUST NOT be worn in general public areas (cafeterias, restrooms, public elevators, office areas, etc.).

EHS can assist in the correct selection of PPE. Again, shorts, tank tops and open toe shoes are forbidden in UNIVERSITY laboratories.

Special handling practices for PPE include:

i. The removal of PPE when going on a coffee break or lunch or dinner break.

ii. The immediate and safe removal and disposal of contaminated PPE.

iii. Frequent hand washing with an appropriate decontaminating soap.

iv. The prohibition of eating, drinking, smoking, chewing gum, removing contact lenses, or applying cosmetics either while in the biohazard area or while wearing potentially contaminated PPE.

6. Laboratory Tasks, Procedures and Safety Protocols must be included. This includes the details important to all laboratory operations, from inception and setup to proper waste management.
7. Inventory of ALL Infectious Agents must be included. Just as a chemical inventory must be included in the CHP, the ECP must include a list of all bloodborne pathogens used in the laboratory. In addition, EHS requires an inventory list of ALL Level 2 human, animal and plant pathogens used in the laboratory.

8. *USDA APHIS Permitting for Plant, Animal, and Veterinary Pathogens.* Any laboratory work involving plant pathogens, veterinary pathogens, or select animal pathogens may require compliance with USDA APHIS regulations (7 CFR Parts 331, 340, 9CFR 121).

Principal Investigators must notify Environmental Health and Safety prior to initiating work
involving:

- Plant pests (e.g. bacteria, fungi, viruses, nematodes, insects)
- Livestock or poultry pathogens
- Veterinary biologics

The University will assist investigators in securing any necessary APHIS, VS, or PPQ permits
prior to the acquisition, transfer, import, or domestic shipment of regulated agents. Documentation of permit approvals must be maintained as part of laboratory biosafety records.

9. Annual Laboratory Specific Training must be conducted once a year or immediately following any changes within the laboratory. This training is conducted by the ECO. All category I and II personnel working in the laboratory must be instructed on changes and revisions, including all special biological safety procedures to be used.

   This training must also include a thorough review of the current operating procedures of the laboratory. Attendance at this session is mandatory. The attendance sign-in sheets must be attached to the ECP.

   NOTE: The ECP site specific training is not the same as the OSHA Bloodborne Pathogen training. EHS conducts OSHA required Hazard Communication, Laboratory Standards, Respirator, and Bloodborne Pathogen standard training once a week and upon request. All new laboratory employees and anyone who has not yet been formally trained must attend all pertinent safety training with EHS. Annual retraining may be completed online from the EHS website at *www.case.edu/ehs*. Anyone who is more than 30 days past due for annual training is required to complete this training in person at EHS. Call EHS at 216-368-2907 for training dates, times and sign-up procedures. New employees must read a copy of the Biosafety Manual and associated documents as listed in the ECP and CHP prior to starting work in the laboratory. New employees must exhibit competency in good laboratory and microbiological techniques prior to start of work in the laboratory.

10. Record Keeping is an integral part of the ECP. This ensures proof that all OSHA regulation requirements have been met.

11. Hepatitis B Virus (HBV) Vaccination must be made available to all employees who are designated as working with bloodborne pathogens (under Categories I & II). These employees must be given the choice of receiving the HBV vaccination at the employer's expense, declining the vaccination, or demonstrating that they have already received the vaccination. An employee who initially declines the vaccination may, at any time during employment, ask for and receive the vaccination at the employer's expense. All employees are to be reminded of vaccination policies during the annual Bloodborne Pathogen Standard retraining. Laboratory personnel must be provided with information regarding vaccines that may be available to protect them against laboratory acquired infection. Under some circumstances, administrators may require immunization as a condition of employment within the biohazard laboratory.

    For instance, the Ohio Department of Health requires that everyone working in the dental school be vaccinated against the HBV. The University requires all medical, nursing and dental students to receive the HBV vaccination prior to their matriculation. Mandatory information provided to employees must include efficacy, side effects, booster schedule, etc. Vaccinations must be provided to employees free of charge and during working hours. Information regarding vaccinations is provided by *University Health Services*.

12. Exposure Incident Counseling must be made available to all employees following a report of an exposure incident. Case Western Reserve University Health services will make immediately available, to any exposed employee, a confidential medical evaluation and follow-up, including at least the following elements:

a. Documentation of the route(s) of exposure, and the circumstances under which the exposure incident occurred.

b. Identification and documentation of the source individual, unless it is established that identification is infeasible or prohibited by state or local law.

c. The source individual's blood will be tested as soon as feasible and after consent is obtained in order to determine HBV and HIV infectivity. If consent is not obtained, Health Services will establish that legally required consent cannot be obtained. When the source individual's consent is not required by law, the source individual's blood, if available, will be tested and the results documented.

d. When the source individual is already known to be infected with HBV or HIV, testing for the source individual's known HBV or HIV status need not be repeated.

e. Results of the source individual's testing will be made available to the exposed employee, and the employee shall be informed of applicable laws and regulations concerning the disclosure of the identity and infectious status of the source individual.

f. The exposed employee's blood will be collected as soon as feasible and tested after consent is obtained.

g. If the employee consents to baseline blood collection but does not give consent at that time for HIV serologic testing, the sample shall be preserved for at least 90 days. If, within 90 days of the exposure incident, the employee elects to have the baseline sample tested, such testing shall be done as soon as feasible.

h. Hepatitis B Prophylaxis will be offered any time an employee is exposed, and Post-Exposure Evaluation and Follow-up must be administered. By law the vaccination series must be paid for by the employer (Case Western Reserve University).

i. The University must ensure that the healthcare professional responsible for the employee's Hepatitis B vaccination is provided a copy of this regulation.

j. The University will ensure that the healthcare professional evaluating an employee after an exposure incident is provided with the following information:

    i. A copy of this regulation.

ii. A description of the exposed employees' duties as they relate to the exposure incident.

iii. Documentation of the route(s) of exposure and circumstances under which exposure occurred.

iv. Results of the source individual's blood testing, if available.

v. All medical records relevant to the appropriate treatment of the employee including vaccination status which are the employer's responsibility to maintain.

k. Health Services will obtain and provide the employee with a copy of the evaluating healthcare professional's written opinion within 15 days of the completion of the evaluation.

l. The healthcare professional's written opinion for Hepatitis B vaccination will be limited to whether Hepatitis B vaccination is indicated for an employee, and if the employee has received such vaccination.

m. The healthcare professional's written opinion for post-exposure evaluation and follow- up will be limited to the following information:

i. That the employee has been informed of the results of the evaluation.

ii. That the employee has been told about any medical conditions resulting from exposure to blood or other potentially infectious materials which require further evaluation or treatment.

n. All other findings or diagnoses shall remain confidential and must not be included in the written report.

13. ECP Review and Revisions are required annually, whether changes in or to the laboratory had occurred or not. This is also required anytime changes do occur, such as personnel, tasks, procedures or material inventories.

14. Housekeeping Considerations (spills, waste disposal, hygiene, etc.) must be included to ensure that the worksite is maintained in a clean and sanitary condition.

a. The ECO shall determine and implement an appropriate written schedule for cleaning and a method of decontamination based upon the location within the facility, the type of surface to be cleaned, the type of soil present, and tasks or procedures being performed in the area.

b. All equipment and environmental and working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials. Contaminated work surfaces shall be decontaminated with an appropriate disinfectant:

i. After completion of procedures.

ii. Immediately or as soon as feasible when surfaces are overtly contaminated or after any spill of blood or other potentially infectious materials.

iii. At the end of the work shift if the surface may have become contaminated since the last cleaning.

c. Protective coverings, such as plastic wrap, aluminum foil, or impervious backed absorbent paper used to cover equipment and environmental surfaces, shall be removed and replaced as soon as feasible when they become overtly contaminated or at the end of the work shift if they may have become contaminated during the shift.

d. All bins, pails, cans, and similar receptacles intended for reuse which have a reasonable likelihood for becoming contaminated with blood or other potentially infectious materials shall be inspected and decontaminated on a regularly scheduled basis and cleaned and decontaminated immediately or as soon as feasible upon visible contamination.

e. Broken glassware which may be contaminated shall not be picked up directly with the hands. It shall be cleaned up using mechanical aids, such as a brush and dustpan, tongs, or forceps.

f. Reusable sharps that are contaminated with blood or other potentially infectious materials shall not be stored or processed in a manner that requires employees to reach by hand into the containers where these sharps have been placed.

15. Additional Requirements for Laboratories using HIV or HBV apply to research laboratories and production facilities engaged in the culture, production, concentration, experimentation, and manipulation of HIV and HBV. These requirements do not apply to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissues, or organs. Additional requirements include:

a. All regulated waste must either be incinerated or decontaminated by a method such as autoclaving, which is known to effectively destroy bloodborne pathogens.

b. Laboratory doors must be kept closed when work involving HIV or HBV is in progress.

c. Contaminated materials that are to be decontaminated at a site away from the work area must be placed in a durable, leakproof, labeled or color-coded container that is closed before being removed from the work area.

d. Access to the work area shall be limited to authorized persons. Written policies and procedures must be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit procedures shall be allowed to enter the work areas and animal rooms.

e. When other potentially infectious materials or infected animals are present in the work area or containment module, a University Caution sign incorporating the universal biohazard symbol with the name of the infectious agent must be posted on all access doors. In addition, the sign must address special requirements for entering the area, names, evening telephone numbers of the PI and list an alternate responsible person.

f. All activities involving other potentially infectious materials shall be conducted in biological safety cabinets or other physical-containment devices within the containment module. No work with potentially infectious materials can be conducted on the open bench.

g. Laboratory coats, gowns, smocks, uniforms, or other appropriate protective clothing shall be used in the work area and animal rooms. Protective clothing shall not be worn outside of the work area and shall be decontaminated before being laundered.

h. Special care shall be taken to avoid skin contact with other potentially infectious materials. Gloves shall be worn when handling infected animals and when making hand contact with other potentially infectious materials is unavoidable.

i. Before disposal, all waste from work areas and from animal rooms must either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

j. Vacuum lines shall be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency, and which are checked routinely and maintained or replaced as necessary.

k. Hypodermic needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle- locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) shall be used for the injection or aspiration of other potentially infectious materials. Extreme caution shall be used when handling needles and syringes. A needle shall not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use.

   The needle and syringe shall be promptly placed in a puncture-resistant container and autoclaved or decontaminated before reuse or disposal.

l. All spills shall be immediately contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with potentially concentrated infectious materials.

m. A spill or accident that results in an exposure incident shall be immediately reported to the laboratory director or an alternate responsible person.

n. The biosafety manual must be periodically reviewed and updated at least annually or more often if necessary. Personnel shall be advised of potential

hazards, shall be required to read instructions on practices and procedures, and shall be required to follow them.

o. Certified BSC (Class I, II, or III) or other appropriate combinations of personal protection or physical containment devices, such as special protective clothing, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals, shall be used for all activities with other potentially infectious materials that pose a threat of exposure to droplets, splashes, spills, or aerosols.

p. When working with biohazardous agents, including bloodborne pathogens, BSCs must be certified when installed, whenever they are moved and at least annually.

q. Each laboratory must contain a facility for hand washing and an eyewash facility which is readily available within the work area.

r. An autoclave for decontamination of regulated waste must be available.

s. The work areas must be separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors is a basic requirement for entry into the work area from access corridors or other contiguous areas.

   Physical separation of the high-containment work area from access corridors or other areas or activities may also be provided through a double-doored clothes-change room (showers may be included), airlock, or other access facility that requires passing through two sets of doors before entering the work area.

t. The surfaces of doors, walls, floors and ceilings in the work area must be water resistant so that they can be easily cleaned. Penetrations in these surfaces must be sealed or capable of being sealed to facilitate decontamination.

u. Each work area must contain a sink for washing hands and a readily available eyewash facility. The sink shall be foot, elbow, or automatically operated and shall be located near the exit door of the work area.

v. Access doors to the work area or containment module must be self-closing.

w. An autoclave for decontamination of regulated waste must be available within or as near as possible to the work area.

x. A ducted exhaust-air ventilation system must be provided. This system must create directional airflow that draws air into the work area through the entry area.

   The exhaust air cannot be recirculated to any other area of the building, must be discharged to the outside, and must be dispersed away from occupied areas and air intakes. The proper direction of the airflow must be verified (i.e., into the work area).

16. Requirements for Laboratories that Modify Protocols, where the general laboratory protocol will not work, the ECO has two choices:

a. For a one-time only procedure, an exemption document can be written. This document must list all modifications to work practices, engineering controls and PPE. It must also state why the normal laboratory protocol cannot be followed. Care must be taken that the modified procedure does not significantly increase the exposure risk to the worker. The exemption document must be placed with the ECP while the procedure is in progress. The document must include the date this procedure will be performed. After the procedure is completed, the document can be removed from the ECP. It is recommended that exemptions be maintained for future reference.

b. For situations involving a reoccurring procedure where the normal ECP is not practical, a separate procedure-specific protocol can be included in the ECP. The procedure- specific plan may be used to redefine work practices, engineering controls or PPE on a permanent basis for that specific procedure. Include this as a permanent part of the ECP. This allows the ECP to be flexible enough to meet the changing needs of the laboratory.

The ECO is free to construct a document that meets the basic criteria outlined here. Hard copies of the ECP, including revisions, must be sent to EHS. An *ECP form* can be downloaded from the EHS website and modified for use in your laboratory.

Facilities

1. Negative pressure tissue culture rooms - In general, a separate tissue culture room provides a higher level of containment for working with potentially airborne recombinant DNA vectors (ex. adenoviral vectors) than the general laboratory. Mechanical ventilation should provide an inward flow of air (negative pressure) without recirculation to spaces beyond the laboratory. This is sometimes referred to as a one-pass ventilation system. Laboratory personnel should verify that the direction of airflow is into the laboratory.
2. Bench Tops - Bench tops should be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat. The work areas should be kept clean and dust free in order to prevent contamination of samples and exposure to agents that can lead to laboratory acquired infections. Bench tops should be disinfected with 10% bleach solution or other appropriate disinfectants following any spill and at least once a month when infrequently used.
3. Laboratory Furniture - Laboratory furniture must be capable of withstanding anticipated loading and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Chairs and other furniture used in laboratory work should be (and must be in BSL3 and BSL4) covered with a solvent impermeable material that can be easily decontaminated.

Posting and Labeling Requirements

Biological hazard posting is used as a means to prevent accidental injury or illness to employees who may be occupationally exposed to biohazardous or potentially biohazardous conditions, equipment or operations which are out of the ordinary, unexpected or not readily apparent. Biological hazard warning labels must be used to identify containers of infectious materials, infectious waste, refrigerators, incubators and/or freezers where biohazards are stored, biosafety cabinets, infectious waste containers, equipment which may be contaminated through normal use of biohazards, laboratory animals (cages) which are potentially infectious or combinations thereof which are potentially contaminated with biohazardous materials. The label must consist of the universal "Biohazard" Symbol and the identity of the biological agent. These labels should be affixed as close as safely possible to the source contaminated or potentially contaminated area.

Signs

The University requires all laboratories to display the formatted University "Caution" sign at all entrances. A biohazard sticker, incorporating the universal biohazard symbol should be placed on the face of these signs.

These signs:

1. Should indicate the biosafety level of the laboratory.
2. Must indicate all infectious agents used in that area.
3. Must include a list of the names and **evening** telephone numbers for the PI and alternate to facilitate contact in case of emergency.

Caution signs and hazard labels can be obtained from the EHS office.

Audit Management

Maintaining accurate records and documentation is a critical part of any Biosafety Program. In order to prove that specific requirements of the Biosafety Program have been accomplished, appropriate documentation must be filed. Documentation is required for the following:

1. Biosafety training (ECP) – Documentation should be available to prove that employees have been trained in the proper use of the specific biohazards with which they work. Documentation should be provided for new personnel and to document retraining. EHS maintains copies of OSHA required training. The laboratory is responsible for proof of CHP and ECP site specific training.
2. Accident Investigation and Injury Illness Recordkeeping – This may be a collaborative effort with EHS and Health Services.
3. Inspection/Audit Reports – Self-inspection and follow-up reports should be maintained for at least one year. EHS performs annual inspections of all University laboratory facilities. These inspections help to ensure that all federal, state and local regulations are in compliance.
4. Highly hazardous organisms (Select Agents BSL3) – incorporate strict requirements covering a multitude of areas, some of which include maintenance of laboratory entry and exit logs, scrupulous inventory control, comprehensive FBI security background check and fingerprinting.

Storage of Biohazardous Materials

Biohazardous material containers must be clearly labeled, as well as the storage space (e.g., freezer, refrigerator), with agent names and the universal biohazard symbol. Emergency

contact info should be visible. Expired or unwanted materials must be decontaminated and disposed of. Long-term storage materials should be inspected annually for damage and replaced or disposed of accordingly. In the event of a freezer malfunction, unsalvageable materials must be autoclaved or chemically disinfected before disposal.

Chapter 2 – Laboratory Practices

General Laboratory Safety

Most of the work performed in any laboratory utilizes the use of general laboratory techniques, good laboratory practices (GLP) and a supporting safety structure. Much of this is covered in *The Case Western Reserve University Laboratory Safety Manual*, and while this manual covers biosafety in the laboratory, general practices of laboratory safety are similarly covered in all safety manuals, concerning use of dangerous or hazardous materials. Common practices include:

1. Persons working in laboratories must be fully aware of the potential hazards to themselves and their co-workers.
2. Eating, drinking, chewing gum, taking medication and applying cosmetics are not permitted in the laboratory.
3. Mouth pipetting is strictly prohibited. Where feasible pipettes should have cotton-plugged tops and must be operated by a mechanical pipetting device.
4. At minimum, a buttoned laboratory coat, proper eye protection and the appropriate gloves must be worn when working in any University laboratory.
5. Non-experimental animals and plants are not permitted in the laboratory.

General Biosafety Procedures

1. All procedures must be performed carefully to minimize the creation of splashes or aerosols. Experimental procedures should be practiced, using non-hazardous materials until the desired technique is mastered.

2. Work surfaces should be decontaminated at least once a day and after any spill of viable or hazardous material. Work surfaces should be decontaminated prior to the start of a procedure to ensure the materials in use and the laboratory workers do not become contaminated.
3. All cultures, stocks, and other regulated wastes must be decontaminated before disposal by an approved decontamination method, such as autoclaving or disinfection with bleach. Materials to be decontaminated outside of the immediate laboratory must be double- bagged in a labeled red biohazard bag, placed in a durable, leak-proof container and closed for transport from the laboratory.
4. Laboratory personnel should receive appropriate immunizations or tests for the agents handled in the laboratory. (Hepatitis B vaccine or TB skin testing).
5. Only needle-locking syringes or disposable syringe-needle units are to be used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. They are to be placed in a red rigid sharps container for disposal.
6. Do not handle broken glassware directly by hand. The glassware should be removed by mechanical means such as a brush and dustpan, tongs, or forceps.
7. All cultures, tissues, or specimens of body fluids must be placed in appropriate container(s) that prevent leakage during collection, handling, processing, storage, transport, or shipping. Secondary specimen containment must be utilized when transporting infectious agents within the campus community.
8. Spills and accidents should be immediately reported to the PI and the EHS office.

Laboratory Biosafety Level Criteria (From the 6th Edition of the BMBL)

There are four biosafety levels for activities involving infectious microorganisms and laboratory animals. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

1. Biosafety Level 1 practices are included in all higher biosafety levels. As the level of biosafety increases, more safety practices are added.
2. Biosafety Level 2 addresses all the practices found in Level 1 but contains added safety practices to accommodate the next higher risk group level organism.
3. Biosafety Level 3 addresses all the practices found in Levels 1 and 2 but contains added safety practices to accommodate the next higher risk group level organism.
4. Biosafety Level 4 addresses all the practices found in Levels 1, 2 and 3, but contains added safety practices to accommodate the highest risk group level organism.

Table I shows an overview of comparisons between all four biosafety level practices.

Biosafety Level (BSL)	**Description**
Biosafety Level 1 (BSL-1)	▪ Suitable for work involving well-characterized agents not known to cause disease consistently in immunocompetent adult humans ▪ Agents present minimal potential hazards to laboratory personnel and the environment
Biosafety Level 2 (BSL-2)	▪ Builds upon BSL-1 ▪ Suitable for work involving agents that post moderate hazards to personnel and the environment
Biosafety Level 3 (BSL-3)	▪ Builds upon BSL-2 ▪ Applicable to facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure
Biosafety Level 4 (BSL-4)	▪ Builds upon BSL-3 ▪ Required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted infections and life- threatening disease that is frequently fatal and for which there are no vaccines or treatments ▪ Required for related agents with unknown risk or route of transmission

Biosafety Level 1

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

Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science. The following standard practices, safety equipment, and facility requirements apply to BSL-1:

1. Standard Microbiological Practices
    a. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
    b. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
    c. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
    d. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
    e. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
        i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
        iii. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
        iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or

forceps. Plasticware should be substituted for glassware whenever possible.

f. Perform all procedures to minimize the creation of splashes and/or aerosols.
g. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
h. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
i. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
j. An effective integrated pest management program is required.
k. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

2. Special Practices – None required
3. Safety Equipment (Primary Barriers and Personal Protective Equipment)
    a. Special containment devices or equipment, such as BSCs, are not generally required.
    b. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

c. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
d. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
    i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
    ii. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

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

Biosafety Level 2

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

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

1. Standard Microbiological Practices
    a. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
    b. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
    c. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. **Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.**
    d. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
    e. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
        i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
        iii. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
        iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
    f. Perform all procedures to minimize the creation of splashes and/or aerosols.
    g. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
    h. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

i. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
j. An effective integrated pest management program is required.
k. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

2. Special Practices
    a. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
    b. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
    c. When appropriate, a baseline serum sample should be stored.
    d. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
    e. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
    f. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

g. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
    i. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
    ii. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
h. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
i. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
j. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

3. Safety Equipment (Primary Barriers and Personal Protective Equipment)
    a. Properly maintained BSCs (preferably Class II), other appropriate PPE, or other physical containment devices must be used whenever:
        i. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
        ii. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
    b. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
    c. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

d. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. **Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should**:
    i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
    ii. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
e. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

4. Laboratory Facilities (Secondary Barriers)
    a. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
    b. 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.
    c. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
    d. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
        i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
        ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
    e. 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.
    f. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
    g. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
    h. An eyewash station must be readily available.

i. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
j. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to ensure proper safety cabinet performance and air system operation must be verified.
k. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate PPE. A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

1. Standard Microbiological Practices
    a. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
    b. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
    c. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
    d. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
    e. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
iii. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

f. Perform all procedures to minimize the creation of splashes and/or aerosols.
g. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
h. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory waste should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
i. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

j. An effective integrated pest management program is required.
k. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur.

   Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection.

   Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

2. Special Practices
   a. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
   b. Laboratory personnel must be provided with medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
   c. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
   d. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
   e. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
   f. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
   g. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
      i. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
      ii. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
   h. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be

reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

i. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

j. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of PPE and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

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

   a. All procedures involving the manipulation of infectious materials must be conducted within a BSC **(**preferably Class II or Class III), or other physical containment devices.

   b. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectants before being laundered. Clothing is changed when contaminated.

   c. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials.

      i. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

   d. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:

      i. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

      ii. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

      iii. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

   e. Eye, face, and respiratory protection must be used in rooms containing infected animals.

4. Laboratory Facilities (Secondary Barriers)
    a. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
    b. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
    c. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be
        i. sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
        ii. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
        iii. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
        iv. Ceilings should be constructed, sealed, and finished in the same general manner as walls. Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.
    d. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
        i. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
        ii. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
    e. All windows in the laboratory must be sealed.
    f. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be

located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

g. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
h. An eyewash station must be readily available in the laboratory.
i. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
    i. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
    ii. The laboratory exhaust air must not be recirculated to any other area of the building.
    iii. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
j. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to ensure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
k. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
l. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
m. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

n. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability.
    i. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
o. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Laundry and Decontamination of Protective Clothing

Potentially contaminated lab coats or protective outer garments such as scrubs are to be handled as little as possible with a minimum of agitation to prevent contamination of the air or of persons handling them. The risk of actual disease transmission from contaminated laundry is low, however care should be taken when handling such clothing. Lab coats should not be taken home for laundering. Professional laundering services are available. Drop off/pick-up points are located at the Service Building and the Wolstein Research Building. The *University Laundry Protocol* can be found at the EHS website.

Use of 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 liquid cultures are pipetted, or when the last drop of an inoculum is blown out. A pipette may become a hazardous piece of equipment if improperly used. A variety of pipetting aids are available, but caution must be exercised with all of them as the use of excessive force when inserting a pipette can fracture the glass and cause serious injury to the hands. The safe pipetting techniques, which follow, are required to minimize the potential for exposure to hazardous materials.

1. Mouth-pipetting is PROHIBITED.
2. If working with biohazardous or toxic fluid, confine pipetting operations to a biosafety cabinet.
3. Always use cotton-plugged pipettes when pipetting biohazardous or toxic materials, even when pipette aids are used.
4. Check your pipette at the beginning of the working day for dust and dirt on the outside. If needed, wipe with 70% ethanol.
5. Do not prepare biohazardous materials by bubbling or expiring air through a liquid with a pipette.
6. Do not forcibly expel biohazardous material out of a pipette.
7. Avoid mixing biohazardous or toxic material by aspirating and discharging through a pipette.
8. When pipetting, avoid accidental release of infectious droplets. Use of a disinfectant soaked towel on the work surface during the procedure, followed by autoclaving the towel after use is an acceptable procedure.
9. Do not discharge material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
10. Prevent cross contamination by using sterilized tips or sterilized filter tips and autoclave the pipette. Avoid contamination to or from fingers by using the tip ejector. Keep the pipette vertical when pipetting in order to prevent the liquid from running into the pipette body.
11. 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 for reuse.
12. Pans or sharps containers for contaminated pipettes should be placed inside the biosafety cabinet.

Syringes and Needles

Syringes and hypodermic needles are dangerous instruments. “Needleless” systems are encouraged whenever possible. The use of needles and syringes should be restricted to procedures for which there is no alternative. Self-sheathing needles are recommended.

Blunt cannulas should be used as alternatives to needles wherever possible (i.e., during procedures such as oral or intranasal animal inoculations). Needles and syringes should never be arbitrarily used as a substitute for pipettes. When needles and syringes must be used, the following procedures are recommended:

1. Use disposable needle locking syringe units whenever possible.
2. When using syringes and needles with biohazardous or potentially infectious agents:
    a. Work in a biosafety cabinet whenever possible.
    b. Wear gloves.
    c. Fill the syringe carefully to minimize air bubbles.
    d. Expel air, liquid and bubbles from the syringe vertically into a cotton pledget moistened with disinfectant.
    e. Do not use a syringe to mix infectious fluid forcefully.
    f. Do not contaminate the needle hub when filling the syringe in order to avoid transferring infectious material to fingers.
    g. Wrap the needle and stopper in a cotton pledget moistened with disinfectant when removing a needle from a rubber-stoppered bottle.
3. Bending, recapping, clipping or removal of needles from syringes is PROHIBITED. If it is essential that a contaminated needle be recapped (e.g. collection of blood for an arterial blood gas analysis), the use of a self-sheathing needle is recommended. As a last resort, the use of an approved mechanical device (capping stand) or the proper "one handed scoop method" must be used. The exact procedure must be written in essay form and be included in the laboratory's ECP. The use of handheld needle clipping devices is PROHIBITED and the devices must be properly discarded.
4. 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.
5. Used disposable needles and syringes must be placed in appropriate red rigid sharps disposal containers and discarded as infectious waste.

Minimization of Aerosol Production

1. Procedures with a high potential for creating aerosols, such as vigorous shaking and vortexing, should be performed in the back third of the biological safety cabinet.
2. Never pour or decant virus suspensions.

3. Avoid vigorous pipetting and mixing. Do not forcibly expel the last drop of virus suspension from a pipette.
4. Discharge pipetted material near the surface of fluid or down the wall of a tube.
5. When opening culture tubes, bottles and flasks, manipulate them slowly.
6. When resuspending liquid cultures, use a swirling action to create a homogeneous suspension with a minimum of aerosolization. Once cultures are resuspended, wait a few minutes to reduce aerosols before opening the container.
7. Do not spray ethanol onto liquid spills, as this will create aerosols. Instead swab the area with a tissue doused in 70% ethanol or 10% bleach.
8. Be careful when using flame-sterilization on utensils, as splattering may occur.

Other devices that result in considerable aerosol production are blenders, ultrasonic disruptors, grinders, lyophilizers and centrifuges. Cell-disrupting and grinding equipment should only be used in a biological safety cabinet when working with biohazardous materials.

Protection of Vacuum Lines

Building vacuum lines must be protected by using two aspirator suction flasks containing bleach in series with a high efficiency particulate air (HEPA) filter installed between the vacuum port and the aspiration flask. This system can isolate and confine infectious materials, preventing fluid and aerosol contamination of vacuum systems, while eliminating hazardous exhaust. Filters are available through laboratory supply catalogs. Contact EHS if you need more information regarding HEPA filters for your vacuum system.

Biological Safety Cabinet Use

The biological safety cabinet (BSC) is the principal engineering control used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

1. To assure sterility inside the cabinet and establish proper airflow for containment, the blower should be turned on at least 10 minutes before infectious materials are to be put in the biosafety cabinet. Check to ensure that the airflow markers fall within the posted safe ranges before working in the hood. Airflow alarms are present on all cabinets. If airflow is incorrect, discontinue work and contact EHS at 216-368-2907. Make sure that all biohazardous materials are properly secured and notify the PI or Laboratory Manager.

2. Before using the BSC, the cabinet should be disinfected so as to avoid accidental exposure to potentially infectious agents and to avoid contamination of cultures. Always keep a bottle of disinfectant (e.g., 10% bleach, 70% ethanol, etc.) in the hood for decontamination and cleaning up spills.
3. All activities involving infectious materials must be conducted in a biological safety cabinet. No work with infectious materials shall be conducted outside the cabinets.
4. NEVER place anything over the front grille of a cabinet. Disrupting the airflow in this manner allows contaminated air from inside the cabinet to blow out of the cabinet into the laboratory or directly at the person sitting at the cabinet. It also allows non sterile air from the room to blow into the cabinet, possibly contaminating the experimental materials within.
5. Materials should be placed in the cabinet so as to not block air flow into the rear grille. Leave a few inches for air to flow around things. Any disruption of the airflow in the cabinet decreases its effectiveness.
6. Before manipulating infectious materials, try to make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less airflow is disrupted.
7. Work should be performed as deeply in the BSC as possible. Infectious agents should not be placed directly adjacent to or on the intake grilles.
8. Any infectious agents that are centrifuged must be contained in screw cap tubes and the aerosol-barrier rotor caps used. The centrifuge container must be loaded and unloaded in the biological safety cabinet.
9. Any waste generated in the biological safety cabinet must be decontaminated or autoclaved for disposal.
10. The biological safety cabinet must be disinfected with 10% bleach or 70% alcohol as appropriate after each use.
11. It is vitally important that natural gas not be used in recirculating hoods. If the gas is left on, an explosive atmosphere can build up and an explosion could occur if ignited.

Steam Sterilization of Reusable Objects/Tools

Reusable objects to be sterilized should first be thoroughly cleaned to remove blood, tissue, food, and other organic residue. Steam sterilization is the best way to achieve inactivation of biological agents. If the item may be damaged by heat, pressure, or moisture, or if it is otherwise not amenable to steam sterilization, please call EHS for advice, at 216-368-2907.

Check to ensure that there are no standing liquids or hazardous chemicals. Place waste in a University standard autoclave bag; autoclave with bag open. After the sterilization cycle, close and seal the bag.

Biohazardous Waste Management

All biohazardous waste must be properly decontaminated and disposed of in accordance with all governmental regulations. According to the Ohio EPA, Ohio Administrative Code 3745-27- 32, documentation of all treated waste must be kept for each treatment for a period of three years, and the log must contain the following entries:

1. Date of treatment.
2. Name and quantity (L or Kg) of material being treated.
3. Name of sterilant/disinfectant being used for treatment.
4. Amount of time material is in contact with sterilant/disinfectant (also include pressure and temperature if autoclaved).
5. Name of individual treating the material.
6. Cycle number for autoclaves.

NEVER GUESS! Call EHS at 216-368-2907 if there are any questions regarding any pathological waste. A copy of the University Waste Chart can be picked up at the EHS office.

Disinfectant/Decontamination Methods

1. Chemical
    a. Sodium Hypochlorite is the most common component used for disinfection and usually consists of a 1:10 dilution of household bleach. Surfaces contaminated with blood or OPIM should be cleaned using a freshly prepared 1:10 dilution of household chlorine bleach solution that is prepared at least daily. The contaminated area should be flooded with the bleach solution and then cleaned up using paper towels. Ten minutes of exposure is required for disinfection. Gloves should be worn during the clean-up procedures. Chlorine bleach can corrode some items and surfaces, so items treated with chlorine should be

rinsed thoroughly. Bleach has a shelf life, and the concentration of sodium hypochlorite diminishes over time. Always make a fresh bleach dilution at least once daily and properly discard bleach over one year from date of manufacture. When using bleach to disinfect biohazardous agents or bloodborne pathogens for disposal, remember to keep an accurate log.

b. Sodium Hydroxide is used for decontamination of mycobacterium tuberculosis and prions. The Petroff method uses a 4% solution for TB, while 40% is used to disinfect prions. Sodium hydroxide is extremely caustic and must be handled correctly, using the proper PPE and neutralization procedures prior to disposal.

c. Formaldehyde is an OSHA-regulated chemical that is a suspected carcinogen, so its use as a disinfectant is not recommended.

d. Iodophors that are registered with the EPA may be effective hard-surface decontaminants when used per manufacturer's instructions, but iodophors formulated as antiseptics are not suitable for use as disinfectants.

e. Peracetic (peroxyacetic) acid and hydrogen peroxide mixtures minimize the negative effects of corrosiveness sometimes seen with chlorine compounds and high concentrations of peracetic acid alone. A limited number of trade-name products containing <0.1% peracetic acid and <1.0% hydrogen peroxide and registered with the EPA as sterilants/disinfectants are available. The benefit of these products is their rapid action and broad-spectrum of germicidal activity, in addition to the reduced corrosiveness.

f. Quaternary ammonium compounds are low-level disinfectants and are not recommended for spills of human blood, blood products, or other potentially infectious materials.

Any chemical disinfectant on the EPA's list of approved tuberculocidal disinfectants (List B) may be used and must be included in the ECP.

2. Autoclaves must operate at a MINIMUM temperature of 121^{o} C (250^{o} F) at a MINIMUM pressure of 15 PSIG for a MINIMUM time of 60 minutes during a treatment cycle (Ohio EPA, Ohio Administrative Code 3745-27-32(D)(1)(a)). These parameters must exist simultaneously (time starts when the proper temperature and pressure are met). Variations of these parameters may be used if it can be validated that the combination of time, temperature, and pressure achieves a performance standard of a four log (base ten) reduction in Bacillus stearothermophilus spores. Other variables to consider include autoclaves utilizing gravity versus vacuum displacement. ALWAYS follow manufacturer's guidelines for proper operation. Autoclaves must not be loaded beyond the total treatable volume of infectious wastes and must be validated periodically, using a noninfectious "dummy" load, to ensure efficacy. Cycle records must be kept and

filed. Only approved materials may be autoclaved. Contact EHS concerning any questions pertaining to autoclave protocols.

Quality assurance and validation testing are required periodically (at least monthly or immediately following any maintenance) to ensure the sterilization cycle is effective. Remember to keep an accurate log of all cycles.

3. Incineration is no longer available at Case Western Reserve University. Biohazardous waste is transported and incinerated off campus by an approved contractor. Waste must be properly packaged and labeled. In most cases, biohazardous waste must be decontaminated by the laboratory generating it prior to being picked up for incineration. This ensures safe transport within the University community. Contact EHS if there are any questions on proper disposal of biohazardous waste.

Biohazardous Liquids

10% bleach is recommended for disinfection of tissue culture waste while 15% bleach is required to inactivate infectious cultures according to state regulations. Cultured human cells that have been treated with an infectious virus or bacteria are required to undergo inactivation using 15% bleach.

After decontamination of biohazardous liquids for a minimum of 30 minutes the liquid can be poured down the sanitary sewer drain with copious amounts of water. Following decontamination, decant all solids (tissue, etc.) prior to pouring. If biohazardous liquids contain other hazardous materials (chemical or radiological, etc.), other additional waste disposal regulations may exist (EPA, ODH,etc.). Even though decontaminated biohazardous liquids can be poured down the drain, the chemical used for decontamination may need to be neutralized or disposed of in another manner. BE CAREFUL to follow proper procedures and check with EHS before proceeding if there are any questions. Remember, an accurate disinfection log must be kept.

Biohazardous Solids

Biohazardous solids include all plastic Petri dishes and plastic tissue culture vessels containing media, whether contaminated or not; cultures and stocks of infectious agents; devices used to transfer, inoculate or mix such agents; paper or cloth contaminated with biohazardous agents. All biohazardous solids must be autoclaved in red bags, marked, sterilized and tagged with the investigators' name and date of sterilization.

The autoclaved waste must remain in the laboratory for pickup by the biowaste crew. Call Customer Service at 216-368-2580 before 4:30 PM for evening pickup. An accurate waste log should be kept.

Biohazardous Regulated SHARPS

1. All syringes, needles, scalpel blades, and razor blades are considered an infectious waste according to the Ohio Infectious Waste Law and, whether contaminated or not, MUST

   always be disposed of in a properly identified rigid container. These containers must also be used for:

   a. Contaminated and uncontaminated lancets

   b. Contaminated and uncontaminated microtome blades

   c. Contaminated and uncontaminated IV tubing with needles attached

   d. Contaminated glass Pasteur pipettes

   e. Contaminated broken and unbroken glass and plastic ware

   f. Any other contaminated items that could potentially puncture a bag

   g. Contaminated disposable plastic pipettes and pipette tips

      Place the sharps in a leak-proof, puncture-proof container with a lid. A fluorescent orange or orange-red label that has a biohazardous symbol in a contrasting color must be affixed to the container. Be sure to label the container with the word "SHARPS". Most biological laboratories use the preferred "red rigid SHARPS container", which can be purchased with proper labeling affixed. Once the containers reach capacity, call Customer Service at 216-368-2580 before 4:30 PM for evening pickup. NEVER OVERFILL SHARPS CONTAINERS.

2. Non-biohazardous plastic pipettes and pipette tips can be placed in a sturdy corrugated box lined with a plastic bag (non-red, orange or yellow). All previous identifying markings must be removed or defaced from the box and the words "Non-biohazardous SHARPS" must be marked. The box must be securely taped. Again, call Customer Service at 216-368-2580 before 4:30 PM for evening pickup.

Mixed Hazardous Waste

Special care must be taken when dealing with biohazardous waste that contains other hazardous materials. For instance, for radioactive biohazardous waste, one of two procedures must be conducted:

1. Radioactivity must be held for 10 half-lives before material can be disposed of as a biohazardous waste.
2. The material must be decontaminated before it can be disposed of as radioactive waste. NOTE: If decontamination utilizes the use of a hazardous chemical, the result will be a chemical/radioactive mixed waste.

When dealing with a biohazardous material containing a hazardous chemical, such as a carcinogen, the material must be decontaminated prior to hazardous chemical disposal.

ALWAYS call EHS for proper waste disposal concerns and include all waste disposal protocols in the CHP and ECP.

Chapter 3- Equipment and Facility Management

Equipment

Autoclaves

Steam Sterilization uses a combination of the variables time, temperature and pressure to eradicate biohazardous organisms. The parameters of these variables depend on the organism, packing density of material the organism is contained within, and environmental conditions such as geographic elevation, ambient temperature and humidity. An autoclave may take 15 minutes to reach peak temperature and pressure, 20 minutes to run the sterilization cycle, and another 15 minutes to reach ambient conditions necessary for the autoclave to be opened safely.

1. An autoclave must be available for the laboratory and must only be operated by personnel who have been properly trained in its use. Improper sterilization could result in laboratory personnel, other personnel involved in disposal of laboratory waste, or the community at large being exposed to potentially infectious agents.
2. Biohazardous materials must not be placed in autoclaves overnight in anticipation of autoclaving the next day.
3. Wrap packages to allow for steam penetration; aluminum foil does not allow steam penetration and should not be used for wrapping.
4. Do not overload the chamber.
5. Avoid overpacking of autoclave bags.
6. Do not seal bags or close bottles and other containers tightly.
7. Do not stack containers.
8. Read the manufacturer's operating manual and post proper work procedures near the autoclave.
9. Never autoclave hazardous chemicals.
10. Strong oxidizers must not be autoclaved with organic materials such as paper, cloth or oil, as this can produce an explosion.
11. Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or simultaneous opening of both doors on a double door autoclave.
12. Open the door slightly to allow escape of steam before unloading.
13. Wear insulated gloves or mitts when unloading.

14. Record all parameters and pertinent information for every cycle (See the Biohazardous Waste Management section of Chapter 3 in this manual).

The changes that are seen on autoclave indicator tapes following an autoclave cycle do not guarantee that the contents of containers are sterile: they indicate only that the tape on the outside of the packages has been exposed to a certain amount of heat or steam. The time required for effective sterilization depends on the size of the load, volumes of liquid and density of materials to be autoclaved. Regular use of either a heat-resistant biological indicator such as Bacillus stearothermophilus, or a chemical indicator should be used to ensure that the cycle in use really achieves sterilization. The indicator is placed in the area least likely to reach sterilizing conditions, such as in the middle of the largest or densest package. A subsequent color change in the chemical indicator, or no color change in the biological indicator, indicates that the load has been exposed to the required conditions for a sufficient length of time.

Manufacturer's suggested instructions must be followed. Vital parameters of validation/QA cycles must be recorded for all test runs.

Centrifuges

Improperly used or maintained centrifuges can present significant hazards to users. Failed mechanical parts can result in release of flying objects, hazardous chemicals and biohazardous aerosols. The high-speed spins generated by centrifuges can create large amounts of aerosol if a spill, leak or tube breakage occurs. To avoid contaminating your centrifuge:

1. Check glass and plastic centrifuge tubes for stress lines; hairline cracks and chipped rims before use. Use unbreakable tubes whenever possible.
2. Avoid filling tubes to the rim.
3. Follow manufacturer's recommendations for tube and rotor maximum safe operation speeds.
4. Aerosol-free (sealed) centrifuge buckets or rotors are required for all centrifuging of infectious specimens and bacteria. Only the correct size tubes should be used in any centrifuge bucket.
5. Buckets should be kept clean and free of broken glass and plastic, and be periodically inspected for wear.
6. Use caps or stoppers on centrifuge tubes. Avoid using lightweight materials such as aluminum foil as caps.
7. Once samples to be centrifuged are prepared, load tubes into buckets inside the biological safety cabinet and seal carefully before moving to centrifuge.

8. After centrifugation, buckets should be opened in a biological safety cabinet to prevent exposure from aerosolized particles. Always visually inspect the rotor for signs of tube leakage prior to opening buckets.
9. Decontaminate the outside of the cups or buckets before and after centrifugation. Inspect O-rings regularly and replace if cracked or dry.
10. Ensure that the centrifuge is properly balanced.

When using high-speed or ultra centrifuges, additional practices should include:

1. Connect the vacuum pump exhaust to a disinfectant trap (See “Protection of Vacuum Lines” in Chapter 3).
2. Record each run in a logbook: keep a record of speed and run time for each rotor.
3. Install a HEPA filter between the centrifuge and the vacuum pump.
4. Never exceed the specified speed limitations of the rotor.
5. Regularly inspect the rotor for contamination, corrosion, or cracks.

The potential for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. Cover all potentially contaminated material spun in a tabletop centrifuge with parafilm so that leakage from an improper seal will not spread into the centrifuge container.

Ultracentrifuge rotors cannot be sealed in this manner but should be constantly monitored for leaks. Opening of all centrifuges must be performed slowly.

Microscopes

Tighten caps on flasks of infectious culture before transporting them to the microscope. Infectious cultures in plates or other containers without tight fitting lids must be carried to the microscope in a tray. When using the hemocytometer to count cells, enclose the hemocytometer in a 70% ethanol-disinfectant petri dish for transport to the microscope.

Disinfect the viewing platform of the microscope after each use.

Microtomes

Microtome blades are extremely sharp and must be handled with great care and stored safely when not in use. If the knife projects beyond the sectioning area, a suitable guard must be fitted. Handling and changing of microtome blades causes many (often serious) injuries, and great care must be exercised when performing these operations. Always carry the knife, in its case,

to the microtome. Never leave the knife on a microtome. After use, always return the knife to its case. Disinfect the microtome by wiping with bleach or sodium hydroxide solution. Slide the ''back'' onto the knife before removing it.

Cryostats

1. Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk of infection. Freezing tissue does not inactivate infectious agents.

2. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material.

3. Gloves should be worn during preparation of frozen sections.

4. Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol.

5. Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.

6. Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after cutting of tissue known to contain infectious substances is cut.

7. Handle knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.

8. Consider all solutions used for staining of potentially infected frozen sections to be contaminated

Water Baths

Pathogenic or nonpathogenic agents may contaminate water baths. It is recommended that either 1 oz. of bleach or 1 oz. of phenolic detergent be added to each gallon of water used in a water bath. Phenolic disinfectants are preferred over bleach, but phenolics must be replenished regularly. Propylene glycol has been used effectively as an alternative to water in cold-water baths. Raise the temperature to 90ºC or higher for 30 minutes once a week for decontamination purposes. Avoid using sodium azide to prevent growth of microorganisms (sodium azide forms explosive compounds with some metals). Thimerosal should also be avoided as a bacteriostat or fungistat as it contains mercury. All forms of mercury are poisonous if absorbed. Treated water must be disposed of as hazardous waste. To prevent electrical shocks, unplug the unit before filling or emptying and have the continuity-to-ground checked on a regular basis.

Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols, which may contain viable microorganisms. To eliminate the spattering and aerosolization associated with flaming of loops, char the material before fully inserting the loop into the flame: i.e., before flaming, hold the loop close to (but not into) the flame.

In addition, the use of a shielded electric incinerator minimizes aerosol production during loop sterilization.

Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators are not available. The loops are semiquantitative and can be used for counting bacteria. Open flames inside biosafety cabinets create airflow turbulence that may compromise sterility and worker protection, and heat buildup may damage the HEPA filters. Open flames are extremely dangerous around flammable materials, such as ethanol, which is often found in biological safety cabinets. Follow these tips for avoiding fires in your biological safety cabinet:

1. Use disposable pre-sterilized loops and spreaders.
2. Replace Bunsen burners with alternative technology such as electric loop sterilizers.
3. Do not use Bunsen burners with pilot flame.
4. Use Bunsen burners that come with excess-temperature protection, flame monitor, and regulated timer.
5. Ensure that the gas supply is clearly labeled inside the cabinet. Inspect your gas lines inside the cabinet before use for kinks, tears, holes, and loose connections and replace worn/damaged lines.
6. Stabilize alcohol containers so that they cannot be tipped over.
7. Reduce the amount of flammable chemicals, equipment and supplies in the biosafety cabinet. Use only enough alcohol for one day's work.
8. Have a "snuffing" lid available in case the alcohol in the cabinet container catches fire. Water is not a good choice for putting out fires in biological safety cabinets.
9. If you smell gas, turn off the exterior gas valve and wait until the gas has fully dissipated before lighting any flames. Remember that a biological safety cabinet recirculates air and vapors may build up to explosive levels inside the cabinet.

Physical and Chemical Hazards

Cryogenic Liquids (Chemical/Physical)

Overview: Cryogenic liquids are gases liquefied at temperatures below -150°C. They are extremely cold and can expand to large volumes of gas, posing specific hazards.

Key Risks:

1. **Burns:**
    - Cryogenic liquids can cause serious frostbite on contact with skin (e.g., liquid nitrogen at -196°C).
    - Always use insulated gloves and protective clothing.
    - Use forceps or tongs to handle cooled objects and special cryobiological containers that can withstand extreme temperatures.
    - Do not seal containers; they require venting to prevent explosion.
2. **Explosion:**
    - Rapid vaporization of cryogenic liquids generates large volumes of gas, creating pressure in sealed containers.
    - Use containers designed for low internal pressure and check for obstructions like frost.
3. **Asphyxiation:**
    - Cryogens are colorless and odorless; they can displace breathable oxygen, leading to suffocation.
    - Always use cryogenic liquids in well-ventilated areas.

Types of Cryogenic Liquids:

- **Inert Gases:** Non-reactive (e.g., nitrogen, helium).
- **Flammable Gases:** Can burn in air (e.g., hydrogen, methane).
- **Oxygen:** Supports combustion; requires separate handling precautions.

Transfer and Handling:

- Use special funnels to minimize splashing and follow container instructions.
- Avoid overfilling and using plastic dipsticks, which can shatter.
- Wear insulated gloves when handling or measuring cryogenic liquids.
- Store containers upright and use roller bases for transport.

Maintenance:

- Regularly check the liquid level (at least weekly) to maintain required temperatures.

- If evaporation rates increase or frost appears, the vacuum system may be compromised. Transfer contents and remove damaged containers from service.

Chapter 4 – Transport/Receipt of Infectious Materials

Procedures for receiving and unpacking infectious materials must be established by the laboratories receiving these materials. Employees who have responsibilities that include the receipt of packages must be given specific instructions regarding the receipt of infectious materials and should be trained and qualified to recognize the hazardous nature of the material being received and recognize whether the material has been packaged, labeled and manifested or documented appropriately. This constitutes an audit function for regulatory compliance. Any given package may receive several such audits and visual inspections along its shipping route.

Receipt of Infectious Materials

Shipments of hazardous materials must be received (generally) by those to whom it is addressed. This is most easily accomplished via a certified carrier such as Federal Express. University Mail Services is certified to accept and transfer hazmat packages from the U.S. Postal Service (USPS). Employees should receive shipments in a designated and secure area of the laboratory. This person should have and utilize all appropriate PPE and containment devices (biological safety cabinet or chemical fume hood). Before accepting any package, the parcel should be carefully inspected for leakage indicated by broken or improperly sealed containers. If the package is rejected (not accepted) due to leakage or other damage, the carrier will work with the shipper to resolve the problem. If the shipment is critical and must be accepted, EHS should be contacted at 216-368-2907, and further activities should be conducted with care in a

containment device. All contaminated materials should be collected for proper disposal through consultation with EHS.

Internal Transport of Biohazardous Materials

Live infectious materials, which are removed from the facility for storage in liquid nitrogen or – 70º C freezers should be stored in non-breakable cryovials. The vials must be surface

decontaminated with 70% ethanol after sealing and then transported to the freezers in non-breakable, impermeable, closed containers (ex. Bio transport Carrier).

When transporting hazardous and infectious materials within University areas (laboratory to laboratory), appropriate secondary containment is required. This ensures isolation and containment should an accident occur. The outside of the secondary container must be clean and free from contaminants, which allows the transfer of materials without the use of PPE. Whenever possible, use the building's service elevators.

External Transport of Biohazardous Materials

All shipments of infectious or diagnostic specimens must be packaged, labeled, and transported in conformance with all applicable local, federal, and international transportation and shipping regulations, including U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA). The law requires that all University employees involved with the transport of these materials be properly trained and certified by EHS. NEVER GUESS if a substance is hazardous or not (even dry ice is regulated). Call EHS at 216-368-2907 with all questions regarding hazardous and infectious materials transport.

Infectious Substance Shipping and Training Requirements

Any personnel involved in packaging, labeling, or shipping biological materials defined as infectious substances (Category A or B), diagnostic specimens, or exempt human/animal specimens must complete current training in:

- U.S. Department of Transportation (DOT) Hazardous Materials Regulations (49 CFR)
- International Air Transport Association (IATA) Dangerous Goods Regulations

Training must be repeated every two years, or sooner if regulations change. Certification records must be maintained and available for inspection. Environmental Health and Safety will assist departments in meeting training requirements.

Chapter 5 – Special Considerations for Recombinant and Synthetic Nucleic Acids

Background/NIH

Experiments which utilize recombinant or synthetic nucleic acids that alter gene expression in cell culture, animals or humans are regulated by the National Institutes of Health, Office of Biotechnology Activities (NIH OBA). These materials are considered to be biohazards by the NIH, Centers for Disease Control (CDC) and CWRU.

Information regarding these regulations can be found in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines).* As described in the NIH Guidelines, such research must be reviewed and approved by the CWRU Institutional Biosafety Committee (IBC). This portion of the CWRU Biosafety Manual addresses these experiments and procedures explicitly.

Risk Assessments/ ECPs

All laboratories utilizing recombinant or synthetic nucleic acids (further referred to as 'recombinant materials') must submit an Exposure Control Plan (ECP) to the Department of Environmental Health and Safety. The specific recombinant materials to be used must be listed in the biohazard inventory. The Risk Group can be determined by consulting Appendix B of the NIH Guidelines. Risk Group determination must be based on the wildtype organism even if the proposed research involves attenuated or defective organisms or single genes from pathogenic organisms. The nature of the wildtype biohazard, any mutated or inserted genes and the ways in which the materials will be manipulated must be considered when performing a risk assessment and making a determination of Biosafety Level Containment (BSL).

The ECP should indicate potential exposure information. If the laboratory works with a pathogenic organism, it is strongly encouraged that the laboratory creates post- exposure plans and shares this information with all lab staff and University Health Services. A hard copy should be available and easily accessible in the laboratory in the event that an exposure was to occur while Health Services is closed. This will allow the University Hospitals Emergency Room staff to treat the injured person.

The ECP must be updated annually or when there is a change in personnel, procedures or biohazardous materials.

General Handling of Recombinant Materials

It is expected that all CWRU Laboratories will follow the recommendations set forth in the CDC's Biosafety in Microbiological and Biomedical Laboratories, 6th Edition (BMBL). The additional general handling requirements are as follows:

1. All procedures must be performed carefully to minimize the creation of splashes or aerosols. Experimental procedures should be practiced, using non-hazardous materials until the desired technique is mastered.

2. Work surfaces should be decontaminated at least once a day and after any spill of viable or hazardous material. Work surfaces should be decontaminated prior to the start of a procedure to ensure the materials in use and the laboratory workers do not become contaminated.

3. All cultures, stocks, and other regulated wastes must be decontaminated before disposal by an approved decontamination method, such as autoclaving or disinfection with bleach. Materials to be decontaminated outside of the immediate laboratory must be double-bagged in a labeled red biohazard bag, placed in a durable, leak-proof container and closed for transport from the laboratory.

4. Laboratory personnel should receive appropriate immunizations or tests for the agents handled in the laboratory. (Hepatitis B vaccine or TB skin testing).

5. Only needle-locking syringes or disposable syringe-needle units are to be used for injections or aspiration of infectious materials.

   Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. They are to be placed in a red rigid sharps container for disposal.

6. Do not handle broken glassware directly by hand. The glassware should be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

7. All cultures, tissues, or specimens of body fluids must be placed in appropriate containers that prevent leakage during collection, handling, processing, storage, transport, or shipping. Secondary specimen containment must be utilized when transporting infectious agents within the campus community.

8. Spills and accidents involving recombinant materials should be immediately reported to the PI, the EHS office and the IBC.

Decontamination and Waste Handling

All materials containing recombinant or synthetic nucleic acids must be properly inactivated and decontaminated prior to disposal. The following are the approved methods for proper decontamination and waste handling of recombinant materials:

1. Chemical

   a. Sodium Hypochlorite is the most common component used for disinfection and usually consists of a 1:10 dilution of household bleach. Surfaces contaminated

with blood or OPIM should be cleaned using a freshly prepared 1:10 dilution of household chlorine bleach solution that is prepared at least daily. The contaminated area should be flooded with the bleach solution and then cleaned up using paper towels. Ten minutes of exposure is required for disinfection. 10% bleach is recommended for disinfection of standard tissue culture waste bleach traps while 15% bleach (with a 20-minute contact time) is required to inactivate infectious cultures. Cultured human cells that have been treated with an infectious virus or bacteria are required to undergo inactivation using 15% bleach.

Gloves should be worn during the clean-up procedures. Chlorine bleach can corrode some items and surfaces, so items treated with chlorine should be rinsed thoroughly. Bleach has a shelf life, and the concentration of sodium hypochlorite diminishes over time. Always make fresh bleach dilution at least once daily and properly discard bleach over one year from date of manufacture. When using bleach to disinfect biohazardous agents or bloodborne pathogens for disposal, remember to keep an accurate log.

b. Sodium Hydroxide is used for decontamination of mycobacterium tuberculosis and prions. The Petroff method uses a 4% solution for TB, while 40% is used to disinfect prions. Sodium hydroxide is extremely caustic and must be handled correctly, using the proper PPE and neutralization procedures prior to disposal.

c. Formaldehyde is an OSHA-regulated chemical that is a suspected carcinogen, so its use as a disinfectant is not recommended.

d. Iodophors that are registered with the EPA may be effective hard-surface decontaminants when used per manufacturer's instructions, but iodophors formulated as antiseptics are not suitable for use as disinfectants.

e. Peracetic (peroxyacetic) acid and hydrogen peroxide mixtures minimize the negative effects of corrosiveness sometimes seen with chlorine compounds and high concentrations of peracetic acid alone. A limited number of trade-name products containing <0.1% peracetic acid and <1.0% hydrogen peroxide and registered with the EPA as sterilants/disinfectants are available. The benefit of these products is their rapid action and broad-spectrum of germicidal activity, in addition to the reduced corrosiveness.

f. Quaternary ammonium compounds are low-level disinfectants and are not recommended for spills of human blood, blood products, or other potentially infectious materials. Any chemical disinfectant on the EPA's list of approved tuberculocidal disinfectants (List B) may be used and must be included in the ECP.

2. Autoclaving utilizes heat, pressure and time to sterilize infectious waste.

   Autoclaves must operate at a MINIMUM temperature of 121^{O} C (250^{O} F) at a MINIMUM pressure of 15 PSIG for a MINIMUM time of 60 minutes during a treatment cycle (Ohio EPA, Ohio Administrative Code 3745-27-32(D)(1)(a)). These parameters must exist simultaneously (time starts when the proper temperature and pressure are met). Variations of these parameters may be used if it can be validated that the combination of time, temperature, and pressure achieves a performance standard of a four log (base ten) reduction in Bacillus stearothermophilus spores. Other variables to consider include autoclaves utilizing gravity versus vacuum displacement. ALWAYS follow manufacturer's guidelines for proper operation. Autoclaves must not be loaded beyond the total treatable volume of infectious wastes and must be validated periodically, using a noninfectious “dummy” load, to ensure efficacy. Cycle records must be kept and filed. Only approved materials may be autoclaved. Contact EHS concerning any questions pertaining to autoclave protocols.

   Quality assurance and validation testing are required periodically (at least monthly or immediately following any maintenance) to ensure the sterilization cycle is effective. Remember to keep an accurate log of all cycles.

3. Incineration is no longer available at Case Western Reserve University. Biohazardous waste including recombinant materials, including transgenic animals and/or animals inoculated with recombinant materials, are transported and incinerated off campus by an approved contractor. Waste must be properly packaged and labeled. In most cases, biohazardous waste must be decontaminated by the laboratory generating it prior to being picked up for incineration. This ensures safe transport within the University community. Contact EHS if there are any questions on proper disposal of biohazardous waste.

Liquids Containing Recombinant Materials

After decontamination of biohazardous liquids containing recombinant materials, the liquid can be poured down the sanitary sewer drain with copious amounts of water. Following decontamination, decant all solids (tissue, etc.) prior to pouring. If biohazardous liquids contain other hazardous materials (chemical or radiological, etc.), other additional waste disposal regulations may exist (EPA, ODH, etc.). Even though decontaminated biohazardous liquids can be poured down the drain, the chemical used for decontamination may need to be neutralized or disposed of in another manner. BE CAREFUL to follow proper procedures and check with EHS before proceeding if there are any questions. Remember, an accurate disinfection log must be kept.

Biohazardous solids that have been used with or contain recombinant materials include all plastic Petri dishes and plastic tissue culture vessels containing media, whether

contaminated or not; cultures and stocks of infectious agents; devices used to transfer, inoculate or mix such agents; paper or cloth contaminated with biohazardous agents. All biohazardous solids must be autoclaved in red bags, marked, sterilized and tagged with the investigators' name and date of sterilization.

The autoclaved waste must remain in the laboratory for pickup by the biowaste crew. Call Customer Service at 216-368-2580 before 4:30 PM for evening pickup. An accurate waste log should be kept.

Regulated Sharps

1. All syringes, needles, scalpel blades, and razor blades are considered an infectious waste according to the Ohio Infectious Waste Law and, whether contaminated or not, MUST always be disposed of in a properly identified rigid container. These containers must also be used for:

    a. Contaminated and uncontaminated lancets

    b. Contaminated and uncontaminated microtome blades

    c. Contaminated and uncontaminated IV tubing with needles attached

    d. Contaminated glass Pasteur pipettes

    e. Contaminated broken and unbroken glass and plastic ware

    f. Any other contaminated items that could potentially puncture a bag

    g. Contaminated disposable plastic pipettes and pipette tips

    Place the sharps in a leak-proof, puncture-proof container with a lid. A fluorescent orange or orange-red label that has a biohazardous symbol in a contrasting color must be affixed to the container. Be sure to label the container with the word "SHARPS". Most biological laboratories use the preferred "red rigid SHARPS container", which can be purchased with proper labeling affixed. Once the containers reach capacity, call Customer Service at 216-368-2580 before 4:30 PM for evening pickup. NEVER OVERFILL SHARPS CONTAINERS.

2. Non-biohazardous plastic pipettes and pipette tips can be placed in a sturdy corrugated box lined with a plastic bag (non-red, orange or yellow). All previous identifying markings must be removed or defaced from the box and the words "Non-biohazardous SHARPS" must be marked. The box must be securely taped. Again, call Customer Service at 216-368-2580 before 4:30 PM for evening pickup.

Mixed Hazardous Waste

Special care must be taken when dealing with biohazardous waste that contains recombinant materials and other hazardous materials. For instance, for radioactive biohazardous waste, one of two procedures must be conducted:

1. Radioactivity must be held for 10 half-lives before material can be disposed of as a biohazardous waste.
2. The material must be decontaminated before it can be disposed of as radioactive waste. NOTE: If decontamination utilizes the use of a hazardous chemical, the result will be a chemical/radioactive mixed waste.

When dealing with a biohazardous material containing a hazardous chemical, such as a carcinogen, the material must be decontaminated prior to hazardous chemical disposal. ALWAYS call EHS for proper waste disposal concerns and include all waste disposal protocols in the CHP and ECP.

Chapter 6 – Spills, Exposures, Reporting and Emergency Response

Emergency Preparedness

The fundamental rule in dealing with a biological spill is to be prepared. Preparation involves identification of the biohazard risks, both actual and potential, that are involved on the site and determining the types of potential spills or emergencies which can occur. In order to prepare for a biohazard spill:

1. Know the properties of the ventilation system serving the laboratory or room, the corridors and the building, in order to accurately predict how aerosols or airborne particles will move.
2. Know where fume hoods and biological safety cabinet exhaust ducting goes after leaving the lab area.
3. Know where biohazard areas are and where biohazardous materials are stored.
4. Assess what hazards could result in the event of a fire, flood, or explosion.
5. Establish evacuation routes and procedures to be used in the event of an emergency with biohazardous materials.
6. Establish rules for safe handling, storage and disposal of biohazardous materials to minimize accidental release and set standards for use to avoid conditions which might lead to an accidental spill.
7. Establish an action plan to be followed should a spill occur. This action plan is an emergency response procedure and consists of a step-by-step procedure to follow if a spill occurs. Spill kit materials should be present in proximity to the area where biohazardous materials are handled.
8. Know the Occupational Medical Services' procedures for reporting and dealing with personnel exposure to biohazardous materials.

Accidents and Exposures

Laboratory personnel who are accidentally exposed to a potentially infectious agent or material should report the incident as soon as possible to the PI and EHS. The PI will see that necessary treatment or health monitoring is obtained without delay.

University Health Services will provide follow-up and counseling on risk of infection and its consequences. The Risk Management Employee Illness or Injury Report form must be completed for all workplace injuries and illnesses.

It is strongly recommended that laboratories working with recombinant materials and other biohazards have written post-exposure procedures in place. These procedures should be on file with University Health Services and a hard copy should be located in an easily accessible location in the laboratory in case the exposure occurs after-hours and the researcher must go to the University Hospitals emergency room for treatment.

Incident Reporting

Spills, accidents, exposures and loss of containment involving recombinant materials may require reporting of the incident to the NIH OBA. Appendix G-II-B-2 of the *NIH Guidelines* states:

> Spills and accidents which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules are immediately reported to the Institutional Biosafety Committee. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

As CWRU does not have recombinant research involving large scale quantities (>10L), Risk Group 4 organisms, Biosafety Level 4 Containment or Risk Group 3 strains of Influenza, the University's additional reporting requirements fall under Section IV-B-2- b-(7) of the NIH Guidelines. This section states:

> Reporting any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OBA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

All incidents involving recombinant materials will therefore be presented to the IBC who will investigate and determine if significant exposure or violations occurred, at which time a report will be filed with the NIH OBA.

First Aid

In the event that a substance enters the mouth, eyes, lungs, or penetrates/comes in contact with the skin follow the instructions below and seek immediate medical attention. (**NOTE:** Always be aware of any special precautions and procedures required to mitigate toxic or infectious responses to materials in use, by reading and understanding the ECP BEFORE starting any work with them.

1. Remove all contaminated clothing and place it in the biological safety cabinet.

2. Warn others of the biohazard.
3. Take a shower or rinse the exposed area with disinfectants.
4. Report the spill to the PI or Laboratory Manager. If an individual is injured during work:
    a. Go to the University Hospital Emergency Room to obtain emergency care.
    b. On-site emergency assistance can be obtained by dialing Protective Services at 216-368-3333.
    c. Persons requiring immediate emergency care should seek it. Preparation of paperwork will be secondary to obtaining prompt medical attention.
5. Post accident serum samples for diagnosis of possible laboratory acquired infection will be coordinated by the PI or Laboratory Manager.
6. The PI or Laboratory Manager should accompany injured personnel to receive a medical evaluation and complete an incident form.
7. The healthcare provider will make an initial assessment of risk.
8. University Health Services will provide follow-up and counseling on risk of infection and its consequences.
9. Personnel working in the laboratory, or who have performed duties in the past 6 months in an area containing infectious materials, must attempt to notify their supervisors before seeking medical attention if they:
    a. Develop a fever greater than 100 ºF; or
    b. Display initial onset symptoms consistent with contraction of the infectious agent used in the laboratory.

Spills inside biological safety cabinet

The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory as long as the spilled material is contained in the biological safety cabinet. A Biosafety Cabinet is designed to contain spills and associated aerosols which are released during work within the cabinet. Provided that the Biosafety Cabinet is operating properly and has been inspected and certified, aerosols produced by a spill should be contained. A spill of biohazardous material should be attended to immediately. Decontamination of the work zone can usually be accomplished by direct application of concentrated liquid disinfectants along with a thorough wipe down procedure. Formaldehyde gas decontamination or vaporized hydrogen peroxide (a service provided by an approved contractor) may be required to treat inaccessible sections of the cabinet interior following a spill. Contact EHS after a major spill to

determine the need for decontamination with formaldehyde or hydrogen peroxide. For spills within a BSC, the following steps should be conducted:

1. Alert people in the immediate area of the spill.

2. Chemical decontamination procedures should be initiated immediately while the biological safety cabinet continues to operate.

3. Contain the spill and decontaminate. All workers using the Biosafety Cabinets should have a supply of absorbent materials and decontaminating agents within the cabinet. This avoids the need to withdraw your arms from within the cabinet should a spill occur and allow you to decontaminate yourself prior to leaving the cabinet.

4. Wear appropriate personal protective equipment during the decontamination procedure. The spill should be covered with paper towels or other absorbent materials soaked with a proven decontamination agent (e.g., 1:10 dilution of bleach containing sodium hypochlorite) for 15 to 20 minutes.

   NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions, decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, with a contact time of at least one hour.

5. Use paper towels to wipe up the spill, working from the edges into the center.

6. Place paper towels in a red or orange biohazard bag containing the proper universal biohazard label.

7. Thoroughly rinse the area with water using clean paper towels or other absorbent materials and dry.

8. Decontaminate equipment and utensils. Items that are not readily or easily surface decontaminated should be carefully placed into autoclave bags and removed for further treatment (e.g., decontamination by autoclaving or other approved methods).

9. Contaminated gloves and clothes must be decontaminated or properly disposed of after decontamination of the area is complete.

10. Remove protective gear. Individuals involved in the spill and clean-up should remove protective clothing (either disposing as biohazardous waste or decontaminating), wash their hands and face with an appropriate decontamination soap, and report to the University's Health Services or the University Hospital Emergency Room for any required evaluation or follow-up.

Spills outside biological safety cabinet

Biological spills outside biological safety cabinets will generate aerosols that can be dispersed in the air throughout the laboratory. Appropriate protective equipment is particularly important in decontaminating spills involving microorganisms.

This equipment includes a lab coat with long sleeves, back-fastening gown or coveralls, disposable gloves, disposable shoe covers, and safety goggles and mask or full-face shield. Use of this equipment will prevent contact with contaminated surfaces and protect eyes and mucous membranes from exposure to splattered materials. For minor spills (less than 10 ml and generating little aerosol) on equipment, laboratory benches, walls, or floors**:**

1. Close laboratory doors and post warning signs to prevent others from entering the laboratory.
2. Thoroughly wash hands and other apparently contaminated areas with soap and water.
3. Put on appropriate personal protective equipment during the decontamination procedure.
4. The spill should be covered with paper towels or other absorbent materials soaked with a proven decontamination agent (e.g., 1:10 dilution of bleach containing sodium hypochlorite) for 15 to 20 minutes.

   NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions, decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, with a contact time of at least one hour.

   Place paper towels in a red or orange biohazard bag containing the proper universal biohazard label.
5. Thoroughly rinse the area with water using clean paper towels or other absorbent materials and dry, and place paper towels in a red or orange biohazard bag containing the proper universal biohazard label.
6. If using an autoclave, BE CAREFUL to avoid strong oxidizers from coming in contact with organic materials (See Point 10 of “Autoclaves” under “Equipment” in Chapter 5).
7. Wash hands and other apparently contaminated areas again with soap and water.

8. Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Contaminated PPE must either be DISPOSED as biohazardous waste or properly decontaminated.

For major Spills (greater than 100 ml or with considerable aerosol):

1. Close laboratory doors and post warning signs to prevent others from entering the laboratory.
2. Leave the biological safety cabinet operating and cultures inside the cabinet.
3. Wash hands and other apparently contaminated areas with soap and water.
4. Report the accident to the PI and to the University Biosafety Officer in EHS (216- 368-2907).
5. If personal clothing is contaminated, remove all outer clothing and place it in the autoclave or container for autoclaving. Put on clean garments.
6. Leave the laboratory for 20 minutes to allow dissipation of aerosols created by the spill.
7. Upon returning to the laboratory to start decontamination, check to ensure that laboratory doors are closed and appropriate signs are displayed. Put on gloves and other protective equipment as previously mentioned above.
8. Place paper towels soaked with decontamination solution over the spill area. Do not pour decontamination solution directly onto the spill in order to avoid additional release of aerosols.
9. Let the decontamination solution / microorganism mixture stand for 30 minutes or longer to allow adequate contact time.
10. Wipe up the spill with the soaked paper towels and properly dispose of towels.
11. Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Contaminated PPE must either be DISPOSED as biohazardous waste or properly decontaminated.

    If using an autoclave, BE CAREFUL that strong oxidizers do not come in contact with organic materials (See Point 10 of “Autoclaves” under “Equipment” in Chapter 5).
12. Thoroughly wash hands, face, and other apparently contaminated areas. Special care in decontamination may be necessary. The PI and/or the University Biosafety Officer may require the collection of sample cultures to determine that the area has been effectively decontaminated.

NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions, decontamination of this infectious agent requires a 40% household bleach solution or a 1N NaOH solution, with a contact time of at least one hour.

Appendix

Definitions

Biohazardous Material – any etiological agent derived from living organisms that pose a risk to the health of other living organisms. This includes, but is not limited to:

1. Pathogens such as parasites, fungi, mycoplasma, bacteria, rickettsia, and viruses, and any of their attendant toxins
2. Certain experiments using recombinant or synthetic nucleic acids
3. Materials of human origin including blood, cell lines (primary or established), tissue, organs, etc.
4. Subviral particles such as prions and viroids

Biosafety – the practice of proper safety management that eliminates or minimizes the risk of exposure when working with biohazardous agents. Biosafety practices include risk assessment, work area monitoring, proper implementation of engineering and administrative controls and proper use of personal protective equipment.

Bloodborne Pathogen - any pathological microorganism that is present in human blood and can cause disease in humans. These include, but are not limited to:

1. HIV- containing cell or tissue cultures, organ cultures, and HIV or HBV containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV, as well as other materials known to be contaminated with HIV or HBV.
2. Any perceived or contaminated "SHARPS", including needles, syringes, scalpel blades and razor blades, even if they have never been in contact with bloodborne pathogens.
3. Other Potentially Infectious Materials (OPIM, see in definitions section).

BSC – Biological Safety Cabinet – an engineering control used when working with biohazardous agents, particularly bloodborne pathogens.

CDC – *Center for Disease Control and Prevention* - one of the major operating components of the United States Department of Health and Human Services.

CHO – Chemical Hygiene Officer – the assigned individual of a laboratory, working with chemicals, who is responsible for writing and implementing the Chemical Hygiene Plan.

CHP – Chemical Hygiene Plan – a written program, prescribed in *29 CFR part 1910.1450 (e)*, developed and implemented by the employer which sets forth procedures, equipment, personal protective equipment and work practices that are capable of protecting employees from the health hazards presented by hazardous chemicals used in that particular workplace.

EHS – *Environmental Health and Safety* of Case Western Reserve University.

ECO – Exposure Control Officer – the individual in a laboratory working with bloodborne pathogens, who is assigned responsibility for writing and implementing the Exposure Control Plan.

ECP – Exposure Control Plan – a written program, developed and implemented by the employer, as prescribed in *29 CFR part 1910.1030 (c)(1)(i)*, which sets forth procedures, equipment, personal protective equipment and work practices that are capable of protecting employees from the health hazards presented by bloodborne pathogens used in that particular workplace.

IBC – Institutional Biosafety Committee - Institutional Biosafety Committees (IBCs) were established under the *NIH Guidelines for Research Involving Recombinant* or Synthetic Nucleic Acids to provide local review and oversight of nearly all forms of research utilizing recombinant materials.

Infectious Substance - a viable microorganism, or its toxin, which causes or may cause disease in humans or animals. These substances include agents listed in *42 CFR 72.3* , and any other agents that cause or may cause severe, disabling, or fatal disease.

Occupational Exposure – reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

OSHA – The Occupational Safety and Health Administration, which is a branch of the United States Department of Labor. This is the governing body that regulates and interprets all aspects of worker safety, including the Hazard Communications Standard and the Bloodborne Pathogen Standard.

Other Potentially Infectious Material (OPIM) – include the following:

- Semen
- Vaginal secretions
- Cerebral spinal fluid
- Synovial fluid
- Pleural fluid
- Pericardial fluid

- Peritoneal fluid
- Amniotic fluid
- Saliva (in dental procedures)
- Any human body fluid that is visibly contaminated with blood
- All human body fluids in situations where it is difficult or impossible to differentiate between human body fluids
- Any unfixed human cells, tissue or organs from a human being, living or dead
- Any animal, animal tissue or animal bedding that is contaminated or infected with any HUMAN pathogen such as HIV or HBV or has been inoculated with cells of human origin.

Parenteral – piercing of mucous membranes or the skin barrier through such events as needlesticks, human or animals' bites, cuts, and abrasions.

PPE – Personal protective equipment, specially designed clothing and equipment that is worn by employees to protect from serious workplace injuries or illnesses resulting from contact with biological, chemical, radiological, physical, electrical, mechanical, or other workplace hazards. Besides face shields, safety glasses, hard hats, and safety shoes, PPE includes a variety of devices and garments such as goggles, coveralls, gloves, vests, earplugs, and respirators.

Primary Barrier – The protection of personnel and the immediate laboratory environment from exposure to infectious agents provided by both good microbiological technique and use of appropriate safety equipment.

Principal Investigator (PI) – University faculty member responsible for the research underway in a laboratory.

Recombinant DNA Molecules – rDNA, molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell; or molecules that result from the replication of those described above.

Secondary Barrier – The protection of the environment external to the laboratory from exposure to infectious materials provided by a combination of facility design and operational practices.

Important Links

The most current version of the Chemical Hygiene and Exposure Control Plans can be found on the EHS website:

https://case.edu/ehs/sites/default/files/2022-11/ECP-traditional-2021.pdf

https://case.edu/ehs/sites/default/files/2023-12/CHP%20revised%2012-5-23.pdf

The Current CDC BSL-2 import inspection checklist:

https://www.cdc.gov/import-permit-program/media/pdfs/2024/08/Import_Permit_Checklist_BSL-2.pdf

Lentivirus and Lentiviral Vector Post Exposure Plan:

https://case.edu/ehs/sites/default/files/2025-04/LentivirusPostExposurePlan.pdf

Biological Spill Response Plan:

https://case.edu/ehs/sites/default/files/2025-04/Biohazardous%20Spill%20and%20Exposure%20Response.pdf

Hepatitis B Declination Form:

https://case.edu/ehs/sites/default/files/2025-04/HepB%20Declination%20Form%202025.pdf