Case Western Reserve University

Laboratory Biosafety Manual

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Chapter 1 - Introduction

General

This biosafety manual is a result of a compilation of material shared by other universities and government regulating entities. This manual does not cover every aspect of biosafety. Important procedures, safety precautions and general rules are covered. The application of Good Laboratory Practices (GLP) and appropriate experimental design, coupled with common sense, should reduce or eliminate potential problems while working in a University laboratory environment.

It is vital that all laboratory personnel maintain sensible laboratory work practices and follow all safety precautions. Knowledge and understanding of the potential dangers of pathogens and other biohazardous materials should always be recognized. Extensive guidelines to biosafety and biological agent information can be found in the CDCs ***Biosafety in Microbiological and Biomedical Laboratories (BMBL)***. These guidelines must be read and understood by all laboratory personnel before any biological work in the laboratory is started. Additionally, all laboratory workers must also be familiar with general laboratory standard practices.

Purpose

This Biosafety Manual covers essential procedures, safety precautions and guidelines for handling biological agents. University laboratories using these agents must follow the procedures in this manual. Some of the material is reiterated throughout this manual, as to be as detailed as possible when referring to a specific topic or issue. The University Biosafety Officer, in Environmental Health and Safety, can be contacted if there are any concerns on categorizing, handling, storing, treating or discarding any biohazardous material**.**

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 Acidsto provide local review and oversight of nearly all forms of research utilizing recombinant materials.
Infectious Substance - a viable micro-organism, 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

Chapter 2 - 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 personnel safety training by EHS, each laboratory employee will be trained by the CHO and the ECO responsible for that laboratory.

http://www.case.edu/finadmin/does/web/Forms/PDFdocs/BBPexposure.pdf**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 700+ laboratories on the campus. Application of the University's polices 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

The PI or his/her designee authorizes access to the laboratory. Persons requesting to use the laboratory or equipment must be advised of potential hazards in the laboratory and all biosafety guidelines as presented in this manual.

Access to the laboratory is restricted when work with infectious agents is in progress, after hours, or when laboratory personnel are not available.

Persons at increased risk of acquiring infection or for whom infection may be unusually hazardous, should not be allowed to work in the laboratory. Included in this category are:

- Minors.

- Individuals who are immunosuppressed, immunodeficient, or undergoing immunosuppressive therapy.

Laboratory Security

Certain biohazardous microorganisms and toxins can be dangerous when used by persons or groups involved in terrorism or other illegal activities. Therefore infectious agents should be secured within the laboratory.

The highest level of security is reserved for materials referred to as Select Agents. Work with these agents require implementation of a Biosecurity Plan. The Case Western Reserve University Select Agent Facilities maintain separate biosafety manuals and security plans.

If a request is received from another institution or corporate entity for a dangerous organism for academic purposes, the PI is responsible for ensuring that the receiving entity is a valid research organization and that the transfer has administrative

approval from both institutions. When a request is received, 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 the use of hazardous chemicals occurs. A "hazardous chemical" is defined as a chemical for which there is evidence that acute or chronic health effects may occur in exposed employees. The CHP should include specific work practices, procedures, and policies to ensure that employees are protected from all potentially hazardous chemicals used. Each laboratory should assign a CHO (usually the PI) to write, review, revise the CHP, and train all workers associated with that laboratory.
Hard copies of the CHP, including revisions, must be sent to EHS. A *CHP form* can be downloaded from the EHS website and modified for use in your laboratory.

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** is required for all personnel who within the normal course of their job duties may be occupationally exposed to bloodborne pathogens. Occupational exposure is 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. **List of Laboratory Employees and their Category** must be included in the plan. By listing all Job Classifications in the work area and classifying the employees into “always”, “sometimes” or “never”, it can be shown that an exposure determination was performed on all employees who could potentially be exposed. All individuals who are designated as working with bloodborne pathogens (under Categories I & II) must attend annual bloodborne pathogen training. This list must be kept current and revised anytime any of the following personnel changes occur:

    a. An employee has been terminated or resigns from the laboratory.

    b. A new employee begins work in the laboratory.

    c. An employee changes duties within the laboratory.

4. **Application of Universal Precautions** was the result of increased epidemiologic knowledge concerning HIV and HBV transmission. In 1985 the Center for Disease Control (CDC) recommended that blood-body fluid precautions be consistently used for all human sources. This approach has come to be known as "universal precautions" and is intended for use in all settings in which the risk of human blood exposure is increased and the infection status of the donor or patient is unknown. The concept of Universal Precautions recognizes that medical history and examination cannot reliably identify all patients infected with HIV or other bloodborne pathogens, thereby dictating certain precautionary measures for all body fluids, irrespective of their source.
    OSHA has adopted complete implementation of universal precautions as a major part of its current regulations. Within the framework of Universal Precautions, three major components are emphasized. They consist of:

    a. Barrier precautions such as gloves, masks/protective eyewear, and gowns/aprons.

    b. Hand washing.

    c. SHARPS precautions. These are discussed in more detail below under work practice controls and PPE.

    The concept of Universal Precautions states that all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens. The University has further adopted the following concepts as "universal" for this campus:

    a. Anything within a red or orange container or a red or orange bag is considered contain bloodborne pathogens, and must be disposed of as such.

    b. All needles, syringes, scalpel blades and razor blades are considered bloodborne pathogens, even if they have never come in contact with biologicals, and must be disposed of in a red rigid sharps container (per Ohio Infectious Wastes Laws).

    c. Anything that cannot be properly identified or labeled will be handled as a bloodborne pathogen for disposal purposes.

    d. The ECO must ensure that all participating employees understand and employ these universal precautions

5. **Hazard Control Methods** must be utilized to protect the worker, 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 a HEPA-filtered, recirculated mass airflow within the work space, 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 a 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, with100% 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 a possibility of rapid increase in concentration

Refer to the *CDC/NIH 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 and illustrated 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 the 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 tanks 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 glove, 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, 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 manufacturers 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, rest rooms, 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. **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.

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

10. **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 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 Service*.

11. **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 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 the following information:

   i. A copy of this regulation.

   ii. A description of the exposed employee's 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.

12. **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.

13. **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 imperviously-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 dust pan, 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.

14. **Additional Requirements for Laboratories using HIV or HBV** applies 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 eye wash 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 eye wash 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).

15. **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 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 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 trainings. 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

All infectious material containers to be stored must be clearly labeled. The storage space (e.g., freezer, refrigerator) must also be labeled with the name of the agent(s) labeled on the universal biohazard symbol. Additional information including contact name and emergency numbers must be visible in case of emergency, i.e., freezer breakdown. Expired and other unwanted material must be properly decontaminated and disposed of. Materials for long-term storage must be annually inspected and each container must be checked for cracks and other

damages and properly disposed or replaced. In the event of a freezer melt-down, all materials that are unable to be salvaged must be properly treated by autoclaving or chemical disinfection prior to disposal.

Chapter 3 - 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 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(s) 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 5th 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. Biosafety Level 1 practices are included in all higher biosafety levels. As the level of biosafety increases, more safety practices are added.

-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.
-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.
-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 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:

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    c. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. 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.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of 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.

B. *Special Practices*
None required.

C. *Safety Equipment (Primary Barriers and Personal Protective Equipment)*
1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
    a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. *Laboratory Facilities (Secondary Barriers)*
1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratories 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:

A. *Standard Microbiological Practices*
1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas.
   Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of 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.

B. *Special Practices*

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

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

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

1. Properly maintained BSCs (preferably Class II), other appropriate PPE, or other physical containment devices must be used whenever:
    a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., 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.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment.
   Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
    a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. ***Laboratory Facilities (Secondary Barriers)***

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available.
9. 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.
10. 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 assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

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:

A. *Standard Microbiological Practices*
1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce

risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

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

6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. 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). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur.
    Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of 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.

B. *Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

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

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

1. 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.
2. 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 disinfectant before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials.
   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.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:
   a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
   b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
   c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. ***Laboratory Facilities (Secondary Barriers)***

1. 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.
2. 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.
3. 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

sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
    a. 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.
    b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
    c. 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 shut downs. 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.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. 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.
    a. 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.
    b. The laboratory exhaust air must not re-circulate to any other area of the building.
    c. 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.
10. 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 assure 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.
11. 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).
12. 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.
13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
14. 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.

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.

15. 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.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission.

Agents with a close or identical antigenic relationship to
agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. Access to the laboratory is controlled by the laboratory supervisor in accordance with institutional policies. There are two models for BSL-4 laboratories:

(1) A *Cabinet Laboratory* where all handling of agents must be performed in a Class III BSC.

(2) A *Suit Laboratory* where personnel must wear a positive pressure protective suit.

BSL-4 Cabinet and Suit Laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment. The following standard and special safety practices, equipment, and facilities apply to BSL-4:

A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. All persons leaving the laboratory must be required to take a personal body shower.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Precautions, including those listed below, must be taken with any sharp items. These include:
    a. 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.
    b. Use of needles and syringes or other sharp instruments should be restricted in the laboratory, except when there is no practical alternative.
    c. Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal or decontamination. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal, located as close to the point of use as possible.

d. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.
8. Decontaminate all wastes before removal from the laboratory by an effective and validated method.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. See Appendix G.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of 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.

B. *Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements in accordance with institutional policies. Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes should be authorized to enter. Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the laboratory must be maintained. While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. Laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves, must be used by all personnel entering the laboratory. All persons leaving the laboratory must take a personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering. After the laboratory has been completely decontaminated, necessary staff may enter and exit without following the clothing change and shower requirements described above.
2. Laboratory personnel and support staff must be provided appropriate occupational medical service including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratoryacquired infections.
3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared. The biosafety manual must be available, accessible, and followed.
5. The laboratory supervisor is responsible for ensuring that laboratory personnel:

a. Demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL-4 containment.
b. Receive appropriate training in the practices and operations specific to the laboratory facility.
c. Receive annual updates or additional training when procedural or policy changes occur.

6. Removal of biological materials that are to remain in a viable or intact state from the laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container.
   These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside BSL-4 containment unless inactivated by a validated method.
7. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
   a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory.
   b. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the laboratory. The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.
   c. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the laboratory supervisor, institutional management and appropriate laboratory personnel as defined in the laboratory biosafety manual. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. Supplies and materials that are not brought into the BSL-4 laboratory through the change room, must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility. The doors of the autoclave are interlocked in a manner that prevents opening of the outer door unless the autoclave has been operated through a decontamination cycle. The doors of a fumigation chamber must be secured in a manner that prevents opening of the outer door unless the fumigation chamber has been operated through a fumigation cycle. Only necessary equipment and supplies should be stored inside the BSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the facility.
11. Daily inspections of essential containment and life support systems must be completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.
12. Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel and other responsible staff according to institutional policies.

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

Cabinet Laboratory

1. All manipulations of infectious materials within the facility must be conducted in the Class III biological safety cabinet. Double-door, pass through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s).
   The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed. The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times. The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings. The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves. Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves. Class III cabinet gloves must be inspected for leaks periodically and changed if necessary. Gloves should be replaced annually during cabinet recertification. The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible. Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups. The Class III cabinet must be certified at least annually.
2. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls must be worn by workers when in the laboratory. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. All protective clothing must be removed in the dirty side change room before showering. Reusable clothing must be autoclaved before being laundered.
3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eyeglasses must be decontaminated before removal through the personal body shower.
4. Gloves must be worn to protect against breaks or tears in the cabinet gloves. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

Suit Laboratory

1. All procedures must be conducted by personnel wearing a one-piece positive pressure suit ventilated with a life support system. All manipulations of infectious agents must be performed within a BSC or other primary barrier system.
   Equipment that may produce aerosols must be contained in devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as needed. 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.
2. Protective laboratory clothing such as scrub suits must be worn by workers before entering the room used for donning positive pressure suits. All protective clothing must be removed in the dirty side change room before entering the personal shower. Reusable laboratory clothing must be autoclaved before being laundered.
3. Inner gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves

should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to personal shower. Dispose of used gloves with other contaminated waste.

4. Decontamination of outer suit gloves is performed during operations to remove gross contamination and minimize further contamination of the laboratory.

D. *Laboratory Facilities (Secondary Barriers)*

Cabinet Laboratory

1. The BSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies. Rooms in the facility must be arranged to ensure sequential passage through an inner (dirty) changing area, a personal shower and an outer (clean) change room prior to exiting the room(s) containing the Class III BSC(s). An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on an uninterrupted power supply (UPS). A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom/airlock must be provided at the containment barrier for the passage of materials, supplies, or equipment.
2. A hands-free sink must be provided near the door of the cabinet room(s) and the inner change room. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC and the inner (dirty) change room must be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved. All penetrations in the internal shell of the laboratory and inner change room must be sealed. Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination. Drains in the laboratory floor (if present) must be connected directly to the liquid waste decontamination system. Services, plumbing or otherwise that penetrate the laboratory walls, floors, ceiling, plumbing or otherwise, must ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter. Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment. Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment of the biological agents in use.
4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.
6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II cabinets should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be

placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if each individual laboratory system is isolated by gas tight dampers and HEPA filters. The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure/directional airflow between adjacent areas within the laboratory. Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory. The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified. Supply air to and exhaust air from the cabinet room, inner change room, and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes. All HEPA filters should be located as near as practicable to the cabinet or laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must to be tested and certified annually. The HEPA filter housings should be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports; and ability to scan each filter assembly for leaks.
10. 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 the 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 assure proper safety cabinet performance and air system operation must be verified. Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s).
    Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.
13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the primary wall. This bioseal must be durable and airtight. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed. Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress must be considered.

Suit Laboratory

1. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies. Rooms in the facility must be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area. Entry into the BSL-4 laboratory must be through an airlock fitted with airtight doors. Personnel who enter this area must wear a positive pressure suit with HEPA filtered breathing air. The breathing air systems must have redundant compressors, failure alarms and emergency backup. A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In the event of an emergency exit or failure of chemical shower system a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed. An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on a UPS. A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment.
2. Sinks inside the suit laboratory should be placed near procedure areas and contain traps and be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion.
   The internal surfaces of this shell must be resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved. All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed. Drains if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents and other service lines must be protected by two HEPA filters in series and have protection against insect and animal intrusion. Services, plumbing or otherwise that penetrate the laboratory walls, floors, ceiling, plumbing or otherwise, must ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter. Decontamination of the entire laboratory must be performed using a validated gaseous or vapor method when there have been significant changes in laboratory usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.
4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Sharp edges and corners should be avoided. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.
6. BSCs and other primary containment barrier systems 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.

7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the BSL-4 laboratory. Two inline HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.
8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if each individual laboratory system is isolated by gas tight dampers and HEPA filters. The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure/directional airflow between adjacent areas within the laboratory. Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory. The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified. Supply air to the laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes. All HEPA filters must be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually. The HEPA filter housings should be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports; and ability to scan each filter assembly for leaks.
10. 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 the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.
12. Liquid effluents from chemical showers**,** sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy. Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.
13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the primary wall. This bioseal must be durable and airtight. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed. Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress should be considered.

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
2	• Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs*: • Laboratory coats; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents PPEs: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of lifethreatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust t, vacuum, and decontamination systems • Other requirements outlined in the text

TABLE I: SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS
From the 5th Edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL)

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 service is 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 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. "Needless" 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 transfer of 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 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 disrupters, 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 Safely 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 air flow 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 nonsterile 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 sterilization cycle, close and seal 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 the are any questions regarding any pathological waste. A copy of the University Waste Chart can be picked up 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 suspect 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° C (250° 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 manufacture'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

After decontamination of biohazardous liquids, 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 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 the 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 4 - Standard Operating Procedures

The laboratory standard operating procedures, which clearly delineate the biohazard/recombinant DNA (rDNA) review process, emergency response procedures, handling of infectious waste, shipment, receipt, and handling of biohazards and a universal precautions policy, must be established.

Specific procedures for individual laboratory operations should be numbered and filed in the laboratories operations manual along with either the CHP for chemical procedures, or the ECP for biological procedures, and referred to in individual laboratory notebooks. Safety precautions should be noted for each procedure.

It is important that all laboratory personnel read and understand all laboratory procedures. This must also be covered in the CHP/ECP annual review.

Chapter 5 - 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 manufactures 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 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 log book: 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 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 hemacytometer to count cells, enclose the hemacytometer in a 70% ethanol-disinfected 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). Thimerosol 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)

Cryogenic Liquids are liquefied gases with boiling points below -150°C at atmospheric pressure. All cryogenic liquids have two properties in common: They are extremely cold, and small amounts of these liquids can expand to very large volumes of gas. The gases released from cryogenic also remain cold and can condense the moisture in the surrounding air forming a visible fog or a liquid air mixture. Cryogenic liquids are frequently used in chemical research laboratories as a system of cooling. There are three major risks associated with the use and handling of cryogens which present potential hazards:

1. **Burns** – Cryogenic liquids are extremely cold. At atmospheric pressure, liquid nitrogen boils at -196°C. Do not allow objects cooled by cryogenic liquids to touch your bare skin. Contact with the skin may cause serious frostbite. Because it is extremely cold, it can freeze human flesh almost instantaneously. Use forceps or tongs to remove straws or canes from the storage container. Protective clothing can reduce the hazards of handling these liquids. Insulated or heavy leather gloves should always be worn when handling any object that has been in contact with cryogens. Loose fitting gloves are recommended so that they may be discarded quickly in the event that any liquid get inside them. Special containers are required. Cryobiological storage containers are specifically designed and constructed to withstand the extreme temperature variances involved in handling liquid cryogens. These special containers should be filled slowly to avoid the expansion stress that occurs as a result of the rapid cooling. Too much stress can damage the container. Do not seal the containers. Remember these liquids have very low boiling points, and vessel pressure needs to be relieved in order to prevent explosion.

2. **Explosion** – Cryogenic liquids produces a large amount of gas. Cryobiological storage containers are designed to function with little or no internal pressure. The use of any tight-fitting stopper or plug that prevents the adequate venting of gas builds up pressure that could severely damage or even burst the container. Even icing or accumulated frost can interfere with proper venting. Therefore, containers should be checked for such obstructions.

3. **Asphyxiation –** Cryogens such as liquid nitrogen are colorless and odorless. Small amounts of liquid can evaporate into very large volumes of gas. For example, one liter of liquid nitrogen vaporizes to 695 liters of nitrogen gas when warmed to room temperature (21°C). It can reduce the concentration of breathable oxygen by displacing it, and can cause suffocation. Since it cannot be detected by sight, taste or smell, it may be inhaled as if it were air. Breathing air that is less than 18% oxygen may cause dizziness, unconsciousness and even death. Therefore, cryogenic liquids must always be stored and used ONLY in areas that are fully ventilated.

Besides the above three risks, specific cryogens can exhibit other dangerous properties. Most cryogenic liquids fall into one of the following three groups:

1. **Inert Gases**: These gases usually do not react chemically with other substances. They do not ignite, burn or support combustion. Examples of this group are nitrogen, helium, neon, argon and krypton.

2. **Flammable Gases**: These cryogens produce a gas that can burn in air. The most common examples are hydrogen, methane and liquefied natural or propane gas.

3. **Oxygen**: Many materials considered as non-combustible can burn in the presence of liquid oxygen. Organic materials can react explosively with liquid oxygen. The hazards and handling precautions of liquid oxygen must therefore be considered separately from other cryogenic liquids.

Transfer cryogenic liquids with care. The primary hazards of transferring these liquids from one container to another are spilling and splashing. Special funnels (with the top partially covered) will reduce splashing. Self pressurizing discharge devices are available. These devices control cryogenic liquid withdrawal (up to two liters per minute for liquid nitrogen).
ALWAYS follow the instructions on containers or accessories when transferring cryogens.
NEVER overfill the containers. Filling above the specified level is likely to produce spillage when the necktube core is replaced. Do not use plastic dipsticks because the extremely low temperatures of cryogenic liquids can cause plastics to become very brittle or even shatter.
NEVER use hollow rods or tubes; the gasification and expansion of the rapidly cooling liquid inside the tube will force liquid to spurt from the top of the tube. Always wear insulated or heavy gloves when measuring.

The eyes can be damaged by exposure to these gases, even when the contact is too brief to affect the skin. Handle containers with care. Containers should always be stored in an upright position. Tipping the container or letting it lie on its side can result in spillage and may damage the container or the materials stored in it. Walking or dragging containers could result in a partial or complete vacuum loss. For containers that cannot be easily and safely carried, a roller base can provide safe and easy movement of containers.

The extremely low temperatures of some cryogens provide the protection of the materials stored in cryobiological storage containers. When all of the cryogenic liquid has evaporated, the temperature inside the container will rise slowly. The rate of evaporation depends upon the age, condition and use pattern of the container. Opening and closing the container or moving it about will reduce its cooling efficiency. Always check the liquid level in containers at least weekly; making sure there is enough in the container to maintain the required temperature mode, and to avoid damage to the ampules, canes, straws or vials stored in the container.
If cryogens evaporate faster than usual or if the container is covered with frost or condensation, the vacuum system may be damaged. In such instances, transfer the contents to another container and remove the damaged one from service.

Ultraviolet (UV) (Physical)
When working properly, UV lights emit radiation at 254 nanometers, which can kill infectious agents on the interior surfaces of the cabinets. This wavelength also happens to induce tumors in laboratory rodents and presents an occupational hazard to laboratory workers.

Visualization of DNA often involves the use of UV light. Ethidium bromide fluoresces under UV light. The lamp used to generate the UV light is usually a mercury arc lamp. The primary

emission of the light is 354 nm. There are other emissions from the visible range (400 nm+) down to and below 254 nm. The most hazardous region for human skin is 270-310 nm. Some mercury arc lamps put out a significant portion (20-30%) of their power in this range. To reduce exposure employees should not use the lamp facing up. While using the lamps, wear a protective face shield and cover exposed skin. The effects of UV are erythema (red skin), photokeratitis (small lacerations of the cornea, or "welder's flash) and skin cancer. When using a UV microscope personnel must wear protective goggles or glasses. In addition, anyone else in the room during such use should also wear similar protective equipment. Reflective surfaces can also redirect UV light with high efficiency. For this reason, be extra careful to check surrounding environment when using UV light experimentally or during a disinfection procedure.

For a complete guide to UV safety, including exposure limits, training, labeling, PPE, and general awareness, and refer to the University Physical Safety Manual.

Infrared (IR) (Physical)

Infrared technology is used in a variety of spectral imaging instruments designed to measure properties in biological tissue. Though the dangers of IR utilized in such instruments are low, precautions should be exercised when using this equipment. Always follow the manufacturer's operations instructions. For a complete guide to IR safety, including exposure limits, training, labeling, PPE, and general awareness, and refer to the University Physical Safety Manual.

Laser (Physical)

Laser Capture Microdissection (LCM) technology offers a method of isolating and removing high purity cell populations from a heterogeneous tissue section, cytological preparation, or live cell culture through direct visualization of the cells. Obtained cell samples can be used for a number of molecular analytical methods. DNA and RNA can easily be extracted and used for PCR, gene expression analysis, and proteomics. There are two general classes of laser-capture microdissection:

1. Infrared (IR) capture systems use photo volatilization of cells surrounding a selected area of tissue. Its laser beam diameter can be a small as 7.5 □m. For IR safety guidelines, refer to the University Physical Safety Manual.

2. Ultraviolet (UV) cutting systems, utilize transfer of laser energy to a thermolabile polymer with formation of a polymer-cell composite. These systems use a much smaller laser beam diameter (0.5□m), than the IR capture systems, making it ideal for precise microdissections of single cells. For UV safety guidelines, refer to the University Physical Safety Manual.

The only possible potential risk of harm is damage to the unprotected eye by direct or reflected beam. There are many safeguards associated with these machines, but the chances of being exposed to other hazards, such as aerosolization is greater. Proper PPE must be worn. Never modify or disassemble these units.

Radiation (Physical)

The University is authorized to use radioactive material by the State of Ohio, which became an "Agreement State" on August 31, 1999. Radioactive material is extensively used in the several hundred biomedical research laboratories on campus. Safe use in compliance with the complex controls and regulations governing the use of radioactivity is the primary goal of the Radiation Safety Program. However, EHS affirms this goal must be realized in a research friendly environment, a philosophy captured by the phrase "low impact compliance".

Although radiation is hazardous, its benefits require use to foster biomedical research, just like the need for electricity and many chemicals in research laboratories. Proper use, while keeping any individual's radiation exposure as low as reasonably achievable (ALARA), is the focus of a balanced approach whereby the risks are proportionate to the return.

Science has not provided a definitive answer regarding the risks associated with exposure to the low levels of radiation biomedical researchers may encounter. International committees, viz., the ICRP, BEIR and UNSCEAR, recommend applying the conservative assumption that any radiation exposure may be harmful and to quantify the risk based on the acute, high dose exposure-affect data available. This "Linear No Threshold" (LNT) model establishes limits for annual radiation exposure. These limits are far above the radiation exposures encountered by biomedical research personnel in our laboratory environment. This is assured by vigilant radiation level monitoring and contamination surveys, verified by a rigorous dosimetry program.

Any place where radioactive material and equipment (X-Ray/Irradiators) is used or stored must be labeled with the international radiation warning symbol and a sign designating the nature of the radiation hazard. For most situations within the University, the sign reads "Caution, Radioactive Materials". The purpose of these postings is for awareness of an invisible hazard. All personnel who work with, or may come into contact with radioactive material are required to be appropriately trained. Those not trained and authorized to be in such a "restricted" area, are forbidden carry out work with radioactive materials or radiation-generating equipment.

For complete radiation safety guidelines, refer to the Case Western Reserve University Radiation Safety Manual.

Electrical (Physical)

Electrical hazards can be present during electrophoresis because electricity is fundamental to the process. In addition to electrical shock, the major hazards of gel electrophoresis are related to the chemical (ethidium bromide) and physical properties (electrical field). DNA is separated using gels (mainly agarose), a buffer solution, and an electric field (80-110 volts). New electrophoresis machines come with UL and CE designations. These pieces of equipment have past stringent tests for electric shock protection. If the electrophoresis machines do not have these approvals, the operator must ensure that no exposed live wires or contact are exposed. A ground fault interrupter (GFI) can be added to automatically shut off the electricity in the event of an electrical fault. The voltages used for electrophoresis are sufficient to cause electrocution. Cover the buffer reservoirs during electrophoresis. Always turn off the power supply and unplug the leads before removing a gel.

Ethidium Bromide (Chemical)

Ethidium bromide is a moderately toxic chemical. It has been shown to be mutagenic. Most suggest handling ethidium bromide as a carcinogen. No skin contact is permitted. When working with ethidium bromide, try to minimize the potential for spills. Where practical, purchase ready-made stock solutions from chemical manufacturers in lieu of mixing your own solutions. If you prefer to mix your own solutions of ethidium bromide, protect yourself by doing this process in a fume hood. Perform all processes that generate ethidium bromide dusts or mists inside the fume hood to minimize inhalation exposures. Prevent accidents by transporting small quantities of ethidium bromide in a secondary container instead of carrying large quantities.

Acrylamide (Chemical)

Acrylamide is a common research laboratory chemical. Widely used as a matrix material for electrophoresis separation procedures, acrylamide gels are a basic separation medium for various biochemical techniques. Thus, familiarity with this material may cause some laboratory personnel to overlook the hazardous nature of this toxic substance. Acrylamide is a powerful central and peripheral nervous system toxicant.

Acute (short-term) exposures to low levels of the monomer can damage nerves and cause effects such as drowsiness, lack of coordination, severe stomach pain, hallucinations, and confusion. Chronic (long-term) exposures can cause severe nerve damage and result in sensory and motor impairment marked by numbness and weakness in the hands and legs, and difficulty walking and speaking. Based upon a number of laboratory and pidemiological studies, the U.S. EPA has classified acrylamide as a probable human carcinogen. All measuring, mixing, and handling of the acrylamide monomer should take place in a chemical fume hood while wearing latex gloves, which extend over the cuffs of the lab coat. Once the monomer has polymerized it is no longer hazardous, however, since there is never 100% polymerization, there will always be a potential for exposure to toxic monomer contamination in acrylamide gels. For this reason polymerized gels should be treated with the same caution as the monomer. Elimination of the hazardous powder is one of the best methods to decrease the risk of acrylamide exposure in the laboratory. Where practical, always purchase pre-mixed acrylamide solutions, which are available from various vendors. These solutions have the added advantage of being specifically designed for each application. There is a high level of purity in these gel formulations, and this provides a high rate of consistency and reproducibility.

Phenol (Chemical)

Phenol is a very caustic organic solvent that is used to extract protein from DNA preparations, and as a disinfectant ingredient for water baths. Phenol can cause skin lesions and be readily absorbed through the skin, whereupon it can affect the central nervous system and cause damage to the liver and kidneys. It is also a mutagen, and there is some evidence that phenol may be a reproductive hazard. When heated, phenol will produce flammable vapors that are highly toxic and explosive. Whenever possible, work with phenol in a chemical fume hood, especially when heating it. Never heat or melt phenol in an incubator, microwave, drying oven, or similar appliance. Prevent phenol from contacting skin by wearing neoprene gloves and a laboratory coat. Change gloves frequently. Wear chemical goggles to protect the eyes. Always wash hands thoroughly after handling phenol, even if gloves are used.

Chloroform (Chemical)

Chloroform is widely used in molecular biology as a solvent in organic extraction. It has been shown that generation of phosgene from chloroform has occurred with or without the exposure to flames, electrical arcs, intense sunlight and hot surfaces. Recently it has been suggested that over time chloroform can break down and form phosgene in older, particulary unstabilized, chloroform containers. Researchers should purchase stabilized chloroform whenever possible. Although amylene is used as a stabilizer, there is evidence that it may not prevent phosgene generation. If unstabilized chloroform must be used, treat it like a peroxide forming compound; dated it when received, use it quickly, and properly discarded it after a year. Unstabilized chloroform, that has been in the laboratory for more than one year, should be discarded it as hazardous waste. Store chloroform in a dark place (cabinet) in an amber bottle to reduce the rate of chloroform decomposition. The following recommended actions should be considered:

1. Unless program requirements prohibit it, purchase only stabilized chloroform.

2. Treat chloroform as a time-sensitive chemical (dated when purchased) and discard it within one year. This is especially true of chloroform that is not stabilized or stabilized with amylene.

3. Store chloroform in a dark place (cabinet) in an amber bottle.

4. Open chloroform containers in a hood and let the headspace vent for a few minutes before bringing the container back into the laboratory. If possible dispense chloroform in the chemical fume hood.

Formaldehyde (Chemical)

Formaldehyde is used primarily as a fixative ingredient to preserve biological tissue. It also acts as a decontaminant, which eradicates most infectious agents. This chemical is a known carcinogen and is on the list of Regulated Chemicals. An OSHA regulation, the OSHA Formaldehyde Standard (29 CFR 1910.1048), sets forth very specific requirements that must be adhered to when working with this substance. Investigators working with formaldehyde must read the CWRU Written Program for Formaldehyde. A copy of the program must be kept with the laboratory's CHP, and appropriate changes made in the work practices and CHP. EHS periodically performs formaldehyde monitoring when needed or requested.

Anesthetic Gas (Chemical)

Anesthetic gases such as isoflurane are used to induce and maintain general anaesthesia by depression of the central nervous system resulting in loss of consciousness. According to OSHA, waste anesthetic gases are anesthetic gases that escape into a surrounding area during medical procedures. To reduce or eliminate exposure to "waste anesthetic gases", EHS will perform risk assessment and hazard determination, followed by suggestions for proper implementation of engineering and administrative controls and personnel protective equipment.

Equipment Maintenance

1. Autoclaves, centrifuges, biological safety cabinets, and fume hoods should undergo regular preventative maintenance by qualified personnel.

2. The airflow must be regularly checked in biological safety cabinets and filters must be changed by qualified personnel. If cabinets are not functioning correctly, EHS will contact an approved contactor to perform maintenance operations. All biosafety cabinets must be tested and certified annually. A Biosafety-Hood Certification Request must be filled out and forwarded to EHS for contractor scheduling.

3. Preventative maintenance records should be kept on all equipment.

4. All equipment should be periodically decontaminated. This not only results in good laboratory practices, but it prevents the spread of contamination to other materials and equipment. All equipment at The University MUST be decontaminated prior to repair, relocation or disposal. EHS must be notified prior to any of these events.

Housekeeping

1. All areas of the laboratory must be kept clean and orderly.

2. Dirt, dust and clutter are safety hazards and are not consistent with acceptable biological research.

3. One or more of he following stock solutions of disinfectants should be maintained at each bench top and biological safety cabinet work area:

   a. 10% bleach (1 part bleach to 9 parts water).

   b. 70% ethanol solution.

   c. Other EPA approved tuberculocidal disinfectant.

4. Vacuum lines should be protected by a disinfectant trap (an aspirator suction flask containing bleach) and a HEPA filter between the vacuum port and the aspiration flask to prevent pathogens from entering the vacuum system.

5. All infectious materials, contaminated plasticware/glassware, and contaminated waste must be disinfected prior to washing or disposal.

6. Contaminated materials are to be placed in red biohazard bags prior to autoclaving.

7. Surfaces are to be decontaminated after each use.

8. The Laboratory Manager or other laboratory personnel should conduct periodic inspections of the laboratory. A copy of a BSL1- 3 Infectious Agent Laboratory Audit Checklist can be found on EHS website at www.case.edu/ehs.

Chapter 6 - Radiation Management

Use of Radioactive Isotopes

All personnel working with radioactive materials should be instructed in the use of necessary safeguards and procedures and all visitors should be informed of pertinent precautions to be taken.

1. Laboratory personnel and visitors should be supplied with the following:

    a. Radiation badges, rings, gloves and lab coat.

    b. Radioactive spill cleaning materials.

2. The authorized user should ensure that every visitor has proper authorization and should recommend that no unnecessary visit to areas involved with radioactive use be made.

3. Radioactive material must not leave the jurisdiction of the authorized user under any circumstances.

4. All areas that are to be used for radioactive work must be clearly labeled with the appropriate radiation hazard signs. Contact the EHS Radiation Safety Office at 216-368-2906 for further information. No radioactive material is to be delivered to an area not properly labeled for radioactive use.

5. Measures should be taken to ensure that no modification of equipment or installations that might lead to unforeseen radiation hazards is made without contacting the Radiation Safety Office.

6. Measures should be taken to ensure that no radioactive material is used by unauthorized personnel who do not have the required training to use radioisotopes. The radiation safety coordinator must keep concise and up-to-date records of usage, inventory, and disposal of radioisotopes.

7. Any accident, unusual incident, or personal injury, must be reported to the Radiation Safety Office, and the authorized user.

8. Whenever personnel are working with radioactive isotopes, the area must be monitored with a Geiger counter and wipe tests, and the person should wear a radiation badge or ring. Note: A Geiger counter will not detect H-3. Therefore, a wipe test must be performed in these areas and results analyzed on a liquid scintillation counter.

9. Radiation emitted from isotopes must be properly shielded, depending on the type of radioactive material that is used.

    a. For high energy beta emitters such as P-32, ½ to ¾ inch Plexiglas © is used as shielding.

    b. Lead is used as shielding when working with gamma emitters such as I-125.

    c. Contact the EHS Radiation Safety Office at 216-368-2906 for all questions regarding shielding.

Disposal of Isotopes

It is imperative to remember that all potentially radioactive infectious waste is considered a mixed hazardous waste, and proper disposal procedures must be followed (See "Mixed Hazardous Waste" in Chapter 3). Please contact the Radiation Safety Office for proper disposal procedures if there are plans on generating infectious radioactive waste.

For complete radiation safety guidelines, refer to the Case Western Reserve University Radiation Safety Manual.

Chapter 7 - Emergency Management

Emergency Procedures

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 hazard 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 should occur. This action plan is the emergency response procedure and consist 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

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.

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 a 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 (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. For spills within a BSC, the following steps should be conducted:

1. Alert people in immediate area of 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 agent within the cabinet. This avoids the need to withdraw your arms from within the cabinet should a spill occur and allows you to decontaminate yourself prior to leaving the cabinet.

4. Wear appropriate personal protective equipment during 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 hypochloride) for 15 to 20 minutes.

   NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions including the select agent bovine spongiform encephalopathy (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for 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 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 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 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 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 hypochloride) for 15 to 20 minutes.

   NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions including the select agent bovine spongiform encephalopathy (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for 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 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.

8. If using an autoclave, BE CARFUL to avoid strong oxidizers from coming in contact with organic materials (See Point 10 of “Autoclaves” under “Equipment” in Chapter 5).

9. Wash hands and other apparently contaminated areas again with soap and water.

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

For major Spills (more than 10 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 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 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 of as biohazardous waste or properly decontaminated.

    If using an autoclave, BE CARFUL 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 including the select agent bovine spongiform encephalopathy (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for at least one hour.

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 MSDS for these dangerous substances 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 disinfectant.

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.

Fire Fighting Procedures

Personal safety is each worker's primary concern in the event of fire.

1. Upon learning of the threat of fire within the building, laboratory personnel will, to the extent possible:

    a. Turn of all gas burners, biological safety cabinets, electric motors, and other electrical equipment.

    b. Place containers of infectious materials into autoclaves, incubators, refrigerators, freezers or other storage areas.

    c. Leave the laboratory as quickly as possible using designated fire evacuation routes.

2. Personnel should be trained by the acting University Fire Marshall in the operation of fire extinguishers.

Reporting and Recordkeeping

Accidents, including spills and injuries, must be reported to the PI and to EHS. Accident report forms must be filled out and filed.

Chapter 8 - 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 or not 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. Biotransport 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 buildings 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.

Chapter 9 – Special Considerations for Recombinant and Synthetic Nucleic Acids

Case Western Reserve University
Department of Environmental Health & Safety

CWRU Biosafety Manual

Special Considerations for Recombinant or Synthetic Nucleic Acids

Background/NIH

Experiments which utilize recombinant or synthetic nucleic acids that alter gene expressing 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 Assesments/ ECPs

All laboratories utilizing recombinant or synthetic nucleic acids (further refered 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 is working 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 Laboratoies, 5th Edition (BMBL). The additional general handling requirements are as follows:

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

10. 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.

11. 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.

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

13. 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.
14. 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.

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

16. 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:

2. 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.

4. Autoclaving utilizes heat, pressure and time to sterilize infectious waste. Autoclaves must operate at a MINIMUM temperature of 121° C (250° 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 manufacture'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.
5. 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 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.

Solids Used with Recombinant Materials

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

3. 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 the 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.
4. 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:

3. Radioactivity must be held for 10 half lives before material can be disposed of as a biohazardous waste.

4. 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.

Spills, Exposures, Reporting and Emergency Response

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, Biosaftey 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.

10. Remove all contaminated clothing and place it in the biological safety cabinet.
11. Warn others of the biohazard.
12. Take a shower or rinse the exposed area with disinfectant.
13. Report the spill to the PI or Laboratory Manager. If an individual is injured during work:
    d. Go to the University Hospital Emergency Room to obtain emergency care.
    e. On-site emergency assistance can be obtained by dialing Protective Services at 216-368-3333.
    f. Persons requiring immediate emergency care should seek it. Preparation of paperwork will be secondary to obtaining prompt medical attention.
14. Post accident serum samples for diagnosis of possible laboratory acquired infection will be coordinated by the PI or Laboratory Manager.
15. The PI or Laboratory Manager should accompany injured personnel to receive a medical evaluation and complete an incident form.
16. The healthcare provider will make an initial assessment of risk.
17. University Health Services will provide follow-up and counseling on risk of infection and its consequences.
18. 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:
    c. Develop a fever greater than 100 ºF; or
    d. 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 a 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:

11. Alert people in immediate area of spill.

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

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

14. Wear appropriate personal protective equipment during 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 hypochloride) for 15 to 20 minutes.

    NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions including the select agent bovine spongiform e**ncephalopathy** (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for at least one hour.

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

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

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

18. 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).

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

20. 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 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**:**

11. Close laboratory doors and post warning signs to prevent others from entering the laboratory.

12. Thoroughly wash hands and other apparently contaminated areas with soap and water.

13. Put on appropriate personal protective equipment during decontamination procedure.

14. 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 hypochloride) for 15 to 20 minutes.

    NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions including the select agent bovine spongiform e**ncephalopathy** (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for at least one hour.

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

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

17. Thoroughly rinse 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.

18. If using an autoclave, BE CARFUL to avoid strong oxidizers from coming in contact with organic materials (See Point 10 of "Autoclaves" under "Equipment" in Chapter 5).

19. Wash hands and other apparently contaminated areas again with soap and water.

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

For major Spills (more than 10 ml or with considerable aerosol):

13. Close laboratory doors and post warning signs to prevent others from entering the laboratory.

14. Leave the biological safety cabinet operating and cultures inside cabinet.

15. Wash hands and other apparently contaminated areas with soap and water.

16. Report the accident to the PI and to the University Biosafety Officer in EHS (216-368-2907).

17. If personal clothing is contaminated, remove all outer clothing and place it in the autoclave or container for autoclaving. Put on clean garments.

18. Leave the laboratory for 20 minutes to allow dissipation of aerosols created by the spill.

19. 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.

20. 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.

21. Let decontamination solution / microorganism mixture stand for 30 minutes or longer to allow adequate contact time.

22. Wipe up the spill with the soaked paper towels and properly dispose of towels.

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

    If using an autoclave, BE CARFUL that strong oxidizers do not come in contact with organic materials (See Point 10 of "Autoclaves" under "Equipment" in Chapter 5).

24. 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 including the select agent bovine spongiform e**ncephalopathy** (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for at least one hour.

Fire Fighting Procedures

Personal safety is each worker's primary concern in the event of fire.

3. Upon learning of the threat of fire within the building, laboratory personnel will, to the extent possible:

    d. Turn of all gas burners, biological safety cabinets, electric motors, and other electrical equipment.

    e. Place containers of infectious materials into autoclaves, incubators, refrigerators, freezers or other storage areas.

    f. Leave the laboratory as quickly as possible using designated fire evacuation routes.

4. Personnel should be trained by the acting University Fire Marshall in the operation of fire extinguishers.

Chapter 10 - Forms

The most current version of the Chemical Hygiene and Exposure Control Plans can be found on the EHS website: https://case.edu/ehs/

SECTION 1910.1030- Appendix A
HEPATITIS-B DECLINATION (MANDATORY)

I understand that due to my occupational exposure to blood or other potentially infectious materials I may be at risk of acquiring hepatitis B virus (HBV) infection. I have been given the opportunity to be vaccinated with hepatitis B vaccine, at no charge to myself. However, I decline hepatitis B vaccination at this time. I understand that by declining this vaccine, I continue to be at risk of acquiring hepatitis B, a serious disease. If in future I continue to have occupational exposure to blood or other potentially infectious materials and I want to be vaccinated with hepatitis B vaccine, I can receive the vaccination at no cost to me.

Name (print): _____

Signature: _____

Case ID (ex. xar123): _____
or
Employee Number: _____

Department: _____

Phone Number: _____

Principal Investigator: _____

Date: _____

BIOSAFETY LEVEL 2 INSPECTION CHECKLIST

These questions are based on the Biosafety Level 2 section of *Biosafety in Microbiological and Biomedical Laboratories, 5th* ed., 2007 pages 31-36

Please place an 'X' in the response box that best describes the laboratory in which work with select agents will be carried out.

N.A. = not applicable. If you mark "N.A.", please provide a brief explanation below that item or on a separate page.

	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Comple Date
	Standard Microbiological Practices							
1	The laboratory supervisor must enforce the institutional policies that control access to the laboratory.							
2	Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.							
3	Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.							
4	Mouth pipetting is prohibited; mechanical pipetting devices must be used.							
5	Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:							

5a	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.							
5b	Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.							
	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Complet
5c	Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.							
5d	Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.							
6	Perform all procedures to minimize the creation of splashes and/or aerosols.							
7	Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.							
8	Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:							
8a	Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.							
8b	Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.							
9	A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.							

10	**An effective integrated pest management program is required. See Appendix G.**							
11	**The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of 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.**							
	Question	**Yes**	**No**	**N.A.**	**Comment**	**Assigned To:**	**Target Date for Completion**	**Complet**

Special Practices

1	**All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.**							
2	**Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.**							
3	**Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.**							
4	**A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.**							
5	**The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.**							
6	**Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.**							

7	Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.							
7a	Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.							
7b	Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.							
8	Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.							
9	Animals and plants not associated with the work being performed must not be permitted in the laboratory.							
10	All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.							
	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Complet
Safety Equipment (Primary Barriers and Personal Protective Equipment)								
1	Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:							
1a	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.							
1b	High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.							

2	**Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., 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.**							
3	**Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.**							
4	**Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:**							
4a	**Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.**							
	Question	**Yes**	**No**	**N.A.**	**Comment**	**Assigned To:**	**Target Date for Completion**	**Complet**
4b	**Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.**							
5	**Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.**							
Laboratory Facilities (Secondary Barriers)								
1	**Laboratory doors should be self-closing and have locks in accordance with the institutional policies.**							
2	**Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.**							

3	**The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.**							
4	**Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.**							
4a	**Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.**							
4b	**Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.**							
5	**Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.**							
6	**BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.**							
7	**Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.**							
8	**An eyewash station must be readily available.**							
	Question	**Yes**	**No**	**N.A.**	**Comment**	**Assigned To:**	**Target Date for Completion**	**Complet**
9	**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.**							

10	**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 assure proper safety cabinet performance and air system operation must be verified.**							
11	**A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).**							

BIOSAFETY LEVEL 3 INSPECTION CHECKLIST

These questions are based on the Biosafety Level 3 section of *Biosafety in Microbiological and Biomedical Laboratories, 5th* ed., 2007 pages 36-43

Please place an 'X' in the response box that best describes the laboratory in which work with select agents will be carried out.

N.A. = not applicable. If you mark "N.A.", please provide a brief explanation below that item or on a separate page.

	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Complet Date
Standard Microbiological Practices								
1	The laboratory supervisor must enforce the institutional policies that control access to the laboratory.							
2	Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.							
3	Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.							
4	Mouth pipetting is prohibited; mechanical pipetting devices must be used.							
5	Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:							
5a	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.							
5b	**Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.**							
5c	**Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.**							
	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Completi
5d	**Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.**							
6	**Perform all procedures to minimize the creation of splashes and/or aerosols.**							
7	**Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.**							
8	**Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. 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). Depending on where the decontamination will be performed, the following methods should be used prior to transport:**							
8a	**Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.**							
8b	**Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.**							
9	**A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for**							

	entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.							
10	**An effective integrated pest management program is required. See Appendix G.**							
11	**The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of 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.**							

Special Practices

1	**All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.**							
2	**Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.**							
3	**Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.**							
	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Completi
4	**A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.**							
5	**The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.**							

6	**Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.**							
7	**Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.**							
7a	**Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.**							
7b	**Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.**							
8	**Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.**							
9	**Animals and plants not associated with the work being performed must not be permitted in the laboratory.**							
10	**All procedures involving the manipulation of infectious materials 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 personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.**							

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

1	**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.**							
2	**Protective laboratory clothing with a solid-front such as tie-back or wrap-around 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 disinfectant before being laundered. Clothing is changed when contaminated.**							

	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Completi
3	**Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. 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.**							
4	**Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:**							
4a	**Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.**							
4b	**Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.**							
4c	**Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.**							
5	**Eye, face, and respiratory protection must be used in rooms containing infected animals.**							
Laboratory Facilities (Secondary Barriers)								
1	**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.**							
2	**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.							
3	**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 sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.**							
3a	**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.**							
	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Completi
3b	**Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.**							
3c	**Ceilings should be constructed, sealed, and finished in the same general manner as walls.**							
	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Completi
3d	**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 shut downs. 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.**							
4	**Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.**							
4a	**Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.**							
4b	**Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.**							
5	**All windows in the laboratory must be sealed.**							

6	**BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.**							
7	**Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.**							
8	**An eyewash station must be readily available in the laboratory.**							
9	**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.**							
9a	**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.**							
9b	**The laboratory exhaust air must not re-circulate to any other area of the building.**							
9c	**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.**							
	Question	Yes	No	N.A.	Comment	Assigned To:	Target Date for Completion	Completi
10	**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 assure 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.**							

11	**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).**							
12	**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.**							
13	**Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.**							
14	**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. 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.**							
15	**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.**							