

# Introduction

The University is committed to providing a safe and healthful learning, teaching and research environment. The goals of the University's biological safety program are to:

- protect staff and students from exposure to infectious agents,
- prevent environmental contamination,
- protect experimental materials,
- comply with federal and local regulations.

The Office of Environmental Health and Safety (OEHS) under the direction of the University's Institutional Biosafety Committee and the Office of the Vice Provost for Research developed the Northeastern University Biological Safety Manual. The manual provides university-wide safety guidelines for those working with biohazards. It outlines general policies and procedures for using and disposing of infectious or potentially infectious materials. Federal and state regulations and guidelines mandate these practices. Updates to this manual are available at Environmental Health and Safety. If procedures currently in practice in your laboratory do not comply with those in this manual, please make the necessary changes to do so. Principal investigators or laboratory supervisors must call the Office of Environmental Health and Safety at 373-2769, if they are uncertain how to categorize, handle, store, treat or discard any biologically derived material.

# **I. Program Administration: Responsibilities and Accountability**

## **A. The Office of the President and Office of the Provost.**

The Office of the President and Office of the Provost have ultimate responsibility for biological safety within the University and must along with other officials, provide support for biological safety.

## **B. The Institutional Biosafety Committee (IBC).**

The IBC is charged by the Vice Provost for Research to formulate policy and procedures related to the use of biohazardous agents, including: human pathogens, oncogenic viruses, other infectious agents and recombinant DNA (rDNA). As mandated by the National Institutes of Health, experiments involving human gene therapy, formation of transgenic animals and the generation of rDNA must be reviewed and approved by the IBC. (See section IIC below). In addition, work with human blood, blood products and tissues are also reviewed and approved by the IBC.

## **C. The Office of Environmental Health and Safety (OEHS):**

- OEHS monitors compliance with University safety policies and procedures regarding potentially infectious and biohazardous materials,
- assists PI's in the selection of laboratory practices, equipment and controls,
- provides technical guidance to all personnel on matters related to laboratory safety,
- develops and conducts appropriate training programs to promote techniques for the safe handling and disposal of biohazardous materials,
- approves the use of biohazardous materials by PI's and sets safety criteria for the handling of those agents,
- investigates all reported accidents which may result in personnel or environmental exposure to biohazardous materials,
- coordinates the off-site treatment of infectious wastes.

## **D. Deans/Department Chairs.**

Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their colleges or departments.

### **E. Principal Investigators (PI's).**

PI's are responsible for identifying potentially infectious and biohazardous materials and carrying out specific control procedures within their own laboratories. This responsibility may not be shifted to inexperienced or untrained personnel. PI's are also responsible for the instruction of students and staff in the potential hazards of biologically derived materials. All protocols involving work with potentially infectious agents must be submitted to OEHS for review and approval. For more information call OEHS at 373-2769.

If an incident occurs in a laboratory which includes violations to established safety control procedures, the IBC may recommend to the Vice Provost for Research that the PI and the entire laboratory group be required to receive or participate in additional specialized training.

### **F. Employees.**

Employees have the responsibility to:

- comply with safety guidelines and procedures required for the task(s) performed,
- report unsafe conditions to the PI, supervisor or OEHS,
- seek guidance from their PI, supervisor or OEHS when they are uncertain how to handle, store or dispose of any hazardous or biohazardous material.

## **II. Biohazards and Potentially Infectious Material**

### **A. Definition:**

Biohazards are infectious agents or biologically derived infectious materials that present a risk or potential risk to the health of humans or animals, either directly through infection or indirectly through damage to the environment. Infectious agents have the ability to replicate and give rise to the potential of large populations in nature when small numbers are released from a controlled situation.

### **B. Categories of biohazards or potentially infectious materials**

#### 1. Human, animal and plant pathogens:

- Bacteria, including those with drug resistance plasmids
- Fungi
- Viruses, including oncogenic viruses
- Parasites

#### 2. All human blood, blood products, tissues and certain body fluids

#### 3. Cultured cells (all human or certain animal) and potentially infectious agents these cells may contain.

4. Allergens
5. Toxins (bacterial, fungal, plant, etc.)
6. Certain recombinant products
7. Clinical specimens
8. Infected animals and animal tissues

## **C. Recombinant DNA (rDNA)**

### **1. Generation of rDNA.**

Experiments involving the generation of rDNA may require registration and approval by the IBC. The National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules is the definitive reference for rDNA research in the United States. There may be experiments which are not covered by the guidelines that do require review and approval by outside agencies before initiation or funding. These experiments are not generally associated with biomedical research but are more common in the agricultural and environmental sciences. If the experimental protocol is not covered by the guidelines, contact the Biosafety Officer at 373-2769 for determination of further review.

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

### **2. Human Gene Therapy**

All protocols involving the generation of rDNA for human gene therapy must be approved locally by the IBC and the Institutional Review Board (IRB) prior to submission to outside agencies and the initiation of experimentation. For more details about IBC approval of human gene therapy protocols, call 373-2769. For information about IRB submissions, call 373-4025.

### **3. Transgenic Animals**

Investigators who create transgenic animals must complete a rDNA registration document and submit it to OEHS for IBC approval prior to initiation of experimentation. In addition, the Institutional Animal Care and Use Committee (IACUC) protocol must be approved by OEHS prior to its being given full approval by the IACUC.

### **4. Transgenic Plants**

Experiments to genetically engineer plants by recombinant DNA methods may require registration with the IBC. The NIH rDNA guidelines provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants.

To obtain a rDNA registration document and a copy of current NIH guidelines, call OEHS at 373-2769.

## **D. Other Potentially Hazardous Biological Materials**

### **1. Human Blood, Blood Products, Body Fluids and Tissues**

Biosafety Level 2 practices and procedures must be followed when handling human blood, blood products, body fluids and tissues because of the infectious agents they may contain. Biosafety Level 2 practices and procedures are consistent with the concept known as Universal Precautions; which requires all specimens of human blood or other potentially infectious materials to be treated as if they are infectious. In 1991, the Occupational Safety and Health Administration (OSHA) promulgated a standard to eliminate or minimize occupational exposure to Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. This federal regulation, Occupational Exposure to Bloodborne Pathogens, mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help control the health risk to employees resulting from occupational exposure to human blood and other potentially infectious materials which may contain these or other specified agents.

Free Hepatitis B vaccination is available to all occupationally at-risk University employees through the Lane Health Center. Mandatory safety training which provides information on protection from occupational exposure to infectious materials is offered by OEHS on a monthly basis university-wide. For more information on training or the availability of free Hepatitis B vaccine, call OEHS at 373-2769.

Investigators using human blood, blood products, body fluids or tissues must complete an Exposure Control Plan, which may be obtained from OEHS. The plan is also available on diskette. The completed plan must be readily available in the laboratory for all workers. In addition, investigators must consult with IRB (373-4025) to ensure that all regulatory requirements relating to the use of human materials or subjects in research are met.

Laboratory personnel (faculty and staff) in HIV or HBV research laboratories must fulfill additional OSHA requirements as follows:

- a. The employee must attend an annual general biosafety training offered by OEHS.
- b. The employee must have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

- c. In the laboratory, the employee must demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory to the satisfaction of the principal investigator/laboratory supervisor before being allowed to work with HIV or HBV.
- d. An employee with no prior experience in handling human pathogens must be trained in the laboratory prior to handling infectious materials. Initial work activities shall not include handling of infectious agents. A progression of work activities will be assigned as techniques are learned and proficiency is developed. Participation in work activities involving infectious agents will be allowed only after proficiency has been demonstrated to the satisfaction of the principal investigator/laboratory supervisor.
- e. The employee must view the video Working Safely with HIV in the Laboratory. A copy of the video is available in the Snell Library for viewing.

## **2. Use of Animals**

The use of animals in research requires compliance with the “Animal Welfare Act” and any state or local regulations covering the care or use of animals. Facilities for laboratory animals used for studies of infectious or non-infectious disease should be physically separate from clinical laboratories and facilities that provide patient care.

Vertebrate animal biosafety level criteria must be adhered to where appropriate. All animal protocols involving the use of rDNA; infectious or transmissible agents; human blood, body fluids or tissues; toxins; carcinogenic, mutagenic, teratogenic chemicals; or physically hazardous chemicals (reactive, explosive, etc.) must be submitted to OEHS for review and approval prior to final approval by the Institutional Animal Care and Use Committee (IACUC). The PI must notify Animal Care Office (ACO) and OEHS in writing prior to initiation of experimentation at Animal Biosafety Level 2 or Animal Biosafety Level 3. IACUC “guidelines” are available from ACO (373-3958). Investigators who are uncertain how to categorize agents should call OEHS (373-2769).

## **3. Tissue Culture/Cell Lines**

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified as the same level as that recommended for the agent.

The Centers for Disease Control and Prevention (CDC) and OSHA recommend that all cell lines of human origin be handled at Biosafety Level 2.

Cell lines which are non-primate or are of normal primate origin, which do not harbor a primate virus, which are not contaminated with bacteria, mycoplasma or fungi and which are well established may be considered Class 1 cell lines and handled at Biosafety Level 1.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until proven to be free of all adventitious agents) and all virus and mycoplasma-containing primate cell lines are classified as Class 2 and should be handled at Biosafety Level 2.

Studies involving suspensions of HIV prepared from T cell lines must be handled at Biosafety Level 3.

#### **4. Guidelines for Preventing the Transmission of Tuberculosis**

Since 1985, the incidence of tuberculosis in the United States has been increasing steadily, reversing a 30 year downward trend. Recently, drug resistant strains of *Mycobacterium tuberculosis* have become a serious concern. Outbreaks of tuberculosis, including drug resistant strains, have occurred in health-care environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of health-care workers have died.

In October 1994, CDC published its “Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities, 1994”. The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at-risk for exposure to tuberculosis. For more information, contact OEHS at 373-2769.

Investigators intending to work with *Mycobacterium tuberculosis* in the laboratory must obtain written approval from OEHS before beginning work. Propagation and manipulation of *Mycobacterium tuberculosis* cultures must be performed at Biosafety Level 3. An agent summary statement for this organism may be found in Appendix A.

#### **5. Use of Vaccinia Virus**

Investigators wishing to use vaccinia virus must obtain written approval to do so from OEHS. Biosafety Level 2 practices and procedures must be followed. Experiments involving the generation of recombinant DNA in vaccinia virus must be registered with the IBC. A Registration Document for rDNA Experiments may be obtained from OEHS by calling 373-2769. The completed document must be returned to the OEHS for IBC approval.

All employees who directly handle cultures or animals contaminated or infected with vaccinia, recombinant vaccinia viruses or other orthopox viruses that infect humans, must be offered small pox vaccine. The Lane Health Center requires that investigators complete a “Request For Small Pox Vaccine” form. The completed form must be returned to the Lane Health Center. Lane will notify individuals when the vaccine is available.

## E. Clinical Laboratories

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at Biosafety Level 2. A primary barrier, such as a biological safety cabinet, should be used:

- when it is anticipated that splashing, spraying or splattering of clinical materials may occur,
- for initial processing of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., *M. tuberculosis*),
- to protect the integrity of the specimen.

All laboratory personnel who handle human source materials are required to comply with the OSHA bloodborne pathogens standard as stated above in IID. Universal precautions must be followed when handling human blood, blood products, body fluids or tissues.

The segregation of clinical laboratory functions and restricting access to specific areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented. An Exposure Control Plan must be completed and be available in the laboratory. Additional recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.

## F. Biological Agents/Biohazard Classification

Biological agents are classified according to risk as follows:

### **Class 1**

Agents of no or minimal hazard under ordinary conditions of handling.

### **Class 2**

Agents of ordinary potential hazard. This class includes agents which may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration but which are contained by ordinary laboratory techniques.

### **Class 3**

Agents involving special hazard or agents derived from outside the United States which require a federal permit for importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment.



#### **Class 4**

Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This class includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

#### **Class 5**

Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.

NOTE: Federally licensed vaccines containing live bacteria or viruses are not subject to these classifications. These classifications are applicable, however, to cultures of the strains used for vaccine production, or further passages of the vaccine strains.

A list of Biological Agents classified according to risk may be found in Appendix A. CDC Agent summary statements on retroviruses (including HIV), hepatitis viruses (including HBV) and *Mycobacterium tuberculosis* may also be found in Appendix A. If the biological agent of interest is not listed, contact OEHS.

### **III. Principles of Biosafety**

#### **A. Containment**

The term “*containment*” is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people and the outside environment to potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

**Primary containment**, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection.

**Secondary containment**, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

**Laboratory Practice and Technique.** The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards, and must be trained and proficient in the practices

and techniques required for handling such material safely. The PI or laboratory supervisor is responsible for providing or arranging for appropriate training of personnel.

Each laboratory should develop an operational manual which identifies specific hazards that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures and hazards associated with the handling of infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The PI is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.

**Safety Equipment (Primary Barriers).** Safety equipment includes biological safety cabinets, enclosed containers and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. More information on BSCs may be found on P. 13,16.

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

**Facility Design (Secondary Barriers).** The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave) and handwashing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory.

## **B. Biosafety Levels.**

There are four biosafety levels (BLs) which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely.

**Biosafety Level 1** is appropriate for work done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. It represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.

**Biosafety Level 2** is applicable to work done with a broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Agents can be used safely on the open bench, provided the potential for producing splashes or aerosols is low. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Procedures with high aerosol or splash potential must be conducted in primary containment equipment such as biosafety cabinets. Primary barriers such as splash shields, face protection, gowns and gloves should be used as appropriate. Secondary barriers such as handwashing and waste decontamination facilities must be available.

**Biosafety Level 3** is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents (i.e., *Mycobacterium tuberculosis*, St. Louis encephalitis virus and *Coxiella burnetii*) include autoinoculation, ingestion and exposure to infectious aerosols. Greater emphasis is placed on primary and secondary barriers to protect personnel in adjoining areas, the community and the environment from exposure to infectious aerosols. For example, all laboratory manipulations should be performed in a biological safety cabinet or other enclosed equipment. Secondary barriers include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.

**Biosafety Level 4** is applicable for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. Primary hazards to workers include respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets and autoinoculation. All

manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment. Isolation of aerosolized infectious materials is accomplished primarily by working in a Class III biological safety cabinet or a full-body, air-supplied positive pressure personnel suit. The facility is generally a separate building or a completely isolated zone within a complex with specialized ventilation and waste management systems to prevent release of viable agents to the environment.

### **C. Vertebrate Animal Biosafety Levels.**

There are four animal biosafety levels, designated Animal Biosafety Level 1 through 4, for work with infectious agents in mammals. The levels are combinations of practices, safety equipment and facilities for experiments on animals infected with agents which produce or may produce human infection. In general, the biosafety level recommended for working with an infectious agent *in vivo* and *in vitro* is comparable.

Animal biosafety levels have been adapted for work in agricultural animals by OEHS and ULAR. The use of any barn for research with infectious agents in farm animals must be approved by OEHS and ULAR prior to experimentation.

Summaries of Biosafety Levels and Animal Biosafety Levels may be found in Tables 1 and 2. Complete descriptions of Biosafety Levels 1 through 3 and Animal Biosafety Levels 1 through 3 may be found in Appendices B and C.

## **IV. Practices and Procedures**

### **A . Administrative Controls**

#### **1. Biohazard Warning Signs and Posting**

Each laboratory must have a room sign that provides safety information to visitors and service personnel. Room signs must contain designations for all laboratory hazards in use within the laboratory (carcinogens, acutely toxic agents, reproductive hazards, biohazards, radioactive materials, lasers and magnetic fields). Contact OEHS for more information.

- a. All areas and laboratories which contain biohazardous agents must be posted with a biohazard sign. The sign must be red/orange in color with a biohazard symbol and lettering in black.
- b. All areas and laboratories which contain biohazardous, radioactive or toxic agents must be posted with signs stating "EATING, DRINKING, SMOKING AND APPLYING COSMETICS ARE PROHIBITED IN THIS AREA."

## **2. Biosafety Levels**

The essential elements of the four biosafety levels for activities involving infectious microorganisms are summarized in Table 1: Recommended Biosafety. In general, Class 1 agents are handled at Biosafety Level 1, Class 2 agents at Biosafety Level 2 and so on. The levels are designated in ascending order, by degree of protection provided to personnel, the environment and the community. Complete descriptions of Biosafety Levels 1 through 3 may be found in Appendix B.

## **3. Vertebrate Animal Biosafety Levels**

There are four animal biosafety levels for experiments on animals infected with agents which produce or may produce human infection. They are summarized in Table 2: Recommended Biosafety Levels for Activities with Infected Vertebrates. As with Biosafety Levels, increasing levels of protection to personnel and the environment are provided as the order ascends. Complete descriptions of Animal Biosafety Levels 1 through 3 may be found in Appendix C.

The City of Boston prohibits Biosafety Level 4 or Animal Biosafety Level 4 facilities.

## **4. Medical Surveillance**

- a. A medical surveillance program will be provided for those personnel having substantial direct animal contact through the Lane Health Center.
- b. Vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risk (local or systemic reactions) will be offered to all clearly identified at-risk personnel, because immuno-prophylaxis may provide an additional level of protection.
- c. A medical surveillance program will be provided through the Lane Health Center for those personnel who are occupationally at-risk of exposure to bloodborne pathogens. The program will include free Hepatitis B vaccination, post-exposure evaluation and follow-up. For a more detailed explanation of this program, consult the University's Exposure Control Plan.

## **B. Engineering Controls**

### **1. Biological safety cabinets (BSCs).**

BSCs are designed to contain aerosols generated during work with infectious material through the use of laminar air flow and high efficiency particulate air (HEPA) filtration. Three types of BSCs (Class I, II and III) are used in microbiological laboratories. Open-fronted Class I and Class II BSCs are partial containment devices which provide a primary barrier offering significant levels of protection to laboratory personnel and to the environment when used in combination with good microbiological techniques.

The Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection. It provides protection to personnel and the environment from contaminants within the cabinet. The Class I BSC does not protect the product from “dirty” room air.

The Class II BSC protects the material being manipulated inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination. It meets requirements to protect personnel, the environment and the product. There are three basic types of Class II BSCs: Type A, Type B and 100% Exhaust. The major differences between the three types may be found in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area.

The gas-tight Class III BSC or glove box, provides the highest attainable level of protection to personnel, the environment and the product. It is the only cabinetry which provides a total physical barrier between the product and personnel. It is for use with *high risk* biological agents and is used when absolute containment of highly infectious or hazardous material is required.

It is important to note that laminar flow clean benches must not be utilized for work with biohazardous or chemically hazardous agents. Clean benches provide product protection by ensuring that the product is exposed only to HEPA-filtered air. They do not provide protection to personnel or the ambient environment.

## **2. Safety equipment**

Safety equipment includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses or goggles. Personal protective equipment (PPE) is often used in combination with BSCs and other devices which contain the biohazardous agents, animals or materials. When it is impractical to work in BSCs, PPE may form the primary barrier between personnel and infectious materials. Examples include certain animal studies, animal necropsy, agent production activities and activities relating to maintenance, service or support of the laboratory facility.

Other safety equipment such as safety centrifuge cups and safety blenders are enclosed containers designed to prevent aerosols from being released during centrifugation or homogenization of infectious material.

Containment controls such as BSCs, safety centrifuge cups and blenders must be used for handling infectious agents that can be transmitted through the aerosol route of exposure. A description of effective use of BSCs and information on other safety equipment may be found in the Recommended Work Practices below.

For more information on proper use and selection of a BSC or other safety equipment, call OEHS at 373-2769.

**C. Recommended Work Practices**

## 1. Pipettes and Pipetting Aids

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

- a. Never mouth pipette. Always use a pipetting aid.
- b. If working with biohazardous or toxic fluid, confine pipetting operations to a biosafety cabinet.
- c. Always use cotton plugged pipettes when pipetting biohazardous or toxic materials, even when safety pipetting aids are used.
- d. Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
- e. Do not forcibly expel biohazardous material out of a pipette.
- f. Never mix biohazardous or toxic material by suction and expulsion through a pipette.
- g. When pipetting, avoid accidental release of infectious droplets. Place a disinfectant soaked towel on the work surface and autoclave the towel after use.
- h. Use “to deliver” pipettes rather than those requiring “blowout”.
- i. Do not discharge material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- j. 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.
- k. Discard contaminated disposable pipettes in an appropriate sharps container.
- l. Pans or sharps containers for contaminated pipettes should be placed inside the biosafety cabinet, if possible.



## 2. Syringes and Needles

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

- a. Use disposable needle locking syringe units whenever possible.
- b. When using syringes and needles with biohazardous or potentially infectious agents:
  1. Work in a biosafety cabinet whenever possible.
  2. Wear gloves.
  3. Fill the syringe carefully to minimize air bubbles.
  4. Expel air, liquid and bubbles from the syringe vertically into a cotton pledget moistened with disinfectant.
  5. Do not use a syringe to mix infectious fluid forcefully.
  6. Do not contaminate the needle hub when filling the syringe in order to avoid transfer of infectious material to fingers.
  7. Wrap the needle and stopper in a cotton pledget moistened with disinfectant when removing a needle from a rubber-stoppered bottle.
- c. Bending, recapping, clipping or removal of needles from syringes is prohibited. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one handed scoop method must be used. The use of needle nipping devices is prohibited and the devices must be discarded as infectious waste.
- d. 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.
- e. Used disposable needles and syringes must be placed in appropriate sharps disposal containers and discarded as infectious waste. (See P. 18, Used Sharps)

## 3. Safe and Effective Use of Biosafety Cabinets

**In general:**

- Make sure your BSC is certified when it is installed or after it is moved, and annually thereafter. (For information on cabinet certification call OEHS at 373-2769). Check the magnehelic gauge regularly for an indication of a problem.
- Understand how your cabinet works.
- Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet, people walking rapidly behind you, and open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.
- Plan your work.
- Minimize the storage of materials in and around the BSC.
- Always leave the BSC running during use.

**Operational directions:**

- Before using, wipe work surface with 70% alcohol. Wipe off each item you need for your procedures and place in cabinet.
- DO NOT place objects over the front air intake grille. DO NOT block the rear exhaust grille.
- Segregate contaminated and clean items. Work from “clean to dirty”.
- Place a pan with disinfectant and/or a sharps container inside the BSC for pipette discard. DO NOT use vertical pipette discard canisters on the floor outside cabinet.
- It is not necessary to flame items. This creates turbulence in airflow and will compromise sterility; heat buildup may damage the filters.
- Move arms slowly when removing or introducing new items into the BSC.
- If you use a piece of equipment that creates air turbulence in the BSC (such as a centrifuge, blender) place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
- Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet.
- Clean up all spills in the cabinet immediately. Wait 10 minutes before resuming work.

- When work is finished-remove all materials and wipe all interior surfaces with 70% alcohol.
- Remove lab coat and wash hands thoroughly before leaving laboratory.

#### 4. Cryostats

Frozen sections on unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents.

Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

#### 5. Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions that include safety precautions should be prominently posted on the unit.

Aerosols are created by practices such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and resuspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.

- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters. (For more information, call OEHS.)
- Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturers' recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.

## 6. Personal Protective Equipment (PPE)

PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. PPE must be provided without cost to personnel. The following PPE is recommended for regular use:

### a. Face Protection

Goggles or safety glasses with solid side shields in combination with masks, or chin length face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face. Information on the availability of low cost prescription safety eyewear may be obtained by calling OEHS at 373-2769. Wearing of contact lenses is inappropriate in the laboratory setting.

### b. Laboratory Clothing

This category includes: laboratory coats, smocks, scrub suits, and gowns. Long sleeved garments should be used to minimize the contamination of skin or street clothes and to reduce shedding of microorganisms from the arms. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or laundered by the facility. Personnel must not launder laboratory clothing at home.

### **c. Gloves**

These must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxics and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required. For assistance in glove selection, call OEHS at 373-2769.

When working with hazardous materials, the lower sleeve and the cuff of the laboratory garment should be overlapped by the glove. A long sleeved glove or disposable arm-shield may be worn for further protection of the garment.

In some instances double gloving may be appropriate. If a spill occurs, hands will be protected after the contaminated outer gloves are removed. Gloves must be disposed of when contaminated, removed when work with infectious materials is completed and not worn outside the laboratory. Disposable gloves must not be washed or reused.

### **d. Respirators.**

In certain instances additional PPE may be required. Respirator selection is based on the hazard and the protection factor required. Personnel who require respiratory protection must contact OEHS for assistance in selection of equipment and training in its proper usage. Contact OEHS for assistance in selection of other personal protective equipment as well.

## **7. Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers**

The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should be used in a BSC when working with biohazardous materials.

**Safety blenders**, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation and to withstand sterilization by autoclaving. If blender rotors are not leakproof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

**Lyophilizers and ampoules.** Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the contents without bubbling and withdraw it into a fresh container. Discard the towel and ampoule top and bottom as infectious waste (See P. 27).

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries. The use of polypropylene tubes eliminates this hazard. These tubes are available dust-free or presterilized and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

## 8. Loop sterilizers and Bunsen burners

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols which may contain viable microorganisms. 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 or gas flames are not available. The loops are semiquantitative and can be used for counting bacteria.

Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter. If a gas burner must be used, one with a pilot light should be selected.

## 9. Laundry

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with blood or other potentially infectious materials should be handled as little as possible and decontaminated, preferably by autoclaving, before being sent to the laundry for cleaning. Appropriate PPE must be worn by employees who handle contaminated laundry.

## 10. Housekeeping

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

Laboratory personnel are responsible to clean laboratory benches, equipment and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keeping the laboratory neat and free of clutter - surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewashes, emergency showers and fire extinguishers must not be blocked.
- Proper disposal of chemicals and wastes - old and unused chemicals should be disposed of promptly and properly. Call OEHS at 373-2769 for details.
- Providing a workplace that is free of physical hazards - aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, avoidance of overloaded electrical circuits and avoidance of the creation of electrical hazards in wet areas.
- Removing unnecessary items on floors, under benches or in corners.
- Properly securing all compressed gas cylinders.
- Never using fume hoods for storage of chemicals or other materials.

### **Practical custodial concerns include:**

- Dry sweeping and dusting which may lead to the formation of aerosols is not permitted.
- The usual wet or dry industrial type vacuum cleaner is a potent aerosol generator and, unless equipped with high efficiency particulate air (HEPA) filter, must not be used in the biological

- research laboratory. Their use is prohibited to protect personnel as well as the integrity of the experiment. Wet and dry units with HEPA filters on the exhaust are available from a number of manufacturers.

## **11. Biohazard Spill Clean-up Procedures**

The following procedures are provided as a guideline to biohazardous spill cleanup.

### **a. Inside the BSC:**

- Wear lab coat, safety glasses and gloves during cleanup.
- Allow cabinet to run during cleanup.
- Apply disinfectant and allow a minimum of 20 minutes contact time.
- Wipe up spillage with disposable disinfectant-soaked cloth.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant-soaked cloth.
- Discard contaminated disposable materials in appropriate biohazardous waste container(s) and autoclave before discarding as infectious waste.
- Place contaminated reusable items in biohazard bags, autoclavable pans with lids or wrap in newspaper before autoclaving and cleanup.
- Expose non-autoclavable materials to disinfectant, 20 minute contact time, before removal from the BSC.
- Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving.
- Run cabinet 10 minutes after cleanup before resuming work or turning cabinet off.

### **b. In the lab, outside the BSC:**

- Clear area of all personnel. Wait for aerosol to settle before entering spill area.
- Remove any contaminated clothing and place in biohazard bag to be autoclaved.
- Wear a disposable gown, safety glasses and gloves during clean-up.
- Initiate cleanup with disinfectant as follows:



1. Soak paper towels in disinfectant and place over spill.
2. Encircle the spill with additional disinfectant being careful to minimize aerosolization while assuring adequate contact.
3. Decontaminate all items within the spill area.
4. Allow 20 minutes contact time to ensure germicidal action of disinfectant.
5. Wipe equipment with 1:10 bleach followed by water then 70% alcohol.
6. Place disposable contaminated spill materials in appropriate biohazardous waste container(s) for autoclaving.
7. Place contaminated reusable items in biohazard bags, autoclavable pans with lids or wrap in newspaper before autoclaving and cleanup.

#### **c. Inside Centrifuge**

- Clear area of all personnel. Wait 30 minutes for aerosol to settle before attempting to clean up spill.
- Wear a lab coat, safety glasses and gloves during cleanup.
- Remove rotors and buckets to nearest biological safety cabinet for cleanup.
- Thoroughly disinfect inside of centrifuge.
- Remove contaminated debris after disinfection, place in appropriate biohazardous waste container(s) and autoclave before disposal as infectious waste.

#### **d. Outside lab, during transport**

- Transport biohazardous material in an unbreakable well-sealed primary container placed inside of a second unbreakable lidded container labeled with the biohazard symbol (cooler, plastic pan or pail).
- Should a spill occur in a public area, do not attempt to clean it up without appropriate personal protective equipment.

- As an interim measure, wear gloves and place paper towels, preferable soaked in disinfectant, directly on spilled materials to prevent spread of contamination. To assure adequate contact, surround the spill with disinfectant, if available, taking care to minimize aerosols.
- Call OEHS at 373-2769 to assist in cleanup.

## D. Decontamination

Decontamination is a term used to describe a process or treatment that renders a medical device, instrument, or environmental surface safe to handle. A decontamination procedure can range from sterilization to simple cleaning with soap and water. Sterilization, disinfection and antisepsis are all forms of decontamination.

**Sterilization** is the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.

**Disinfection** eliminates virtually all pathogenic non-sporeforming microorganisms but not necessarily all microbial forms on inanimate objects (work surfaces, equipment, etc.). Effectiveness is influenced by the kinds and numbers of organisms, the amount of organic matter, the object to be disinfected and chemical exposure time, temperature and concentration.

**Antisepsis** is the application of a liquid antimicrobial chemical to skin or living tissue to inhibit or destroy microorganisms. It includes swabbing an injection site on a person or animal and hand washing with germicidal solutions. Although some chemicals may be utilized as either a disinfectant or an antiseptic, adequacy for one application does not guarantee adequacy for the other. Manufacturers' recommendations for appropriate use of germicides should always be followed.

### 1. General Procedures

- All infectious materials and all contaminated equipment or apparatus should be decontaminated before being washed, stored or discarded. Autoclaving is the preferred method. Each individual working with biohazardous material should be responsible for its proper handling.
- Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.
- Autoclaves should not be operated unattended or by untrained personnel.
- 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.
- Dry hypochlorites, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth or oil:

OXIDIZER + ORGANIC MATERIAL + HEAT = MAY PRODUCE AN EXPLOSION.

## 2. Methods

There are four main categories of physical and chemical means of decontamination. They are heat, liquid disinfection, vapors and gases and radiation. Each category is discussed briefly below.

### a. Heat

1. **Wet heat** is the most dependable method of sterilization. Autoclaving (saturated steam under pressure of approximately 15 psi to achieve a chamber temperature of at least 250° F for a prescribed time) is the most convenient method of rapidly achieving destruction of all forms of microbial life. In addition to proper temperature and time, prevention of entrapment of air is critical to achieving sterility. Material to be sterilized must come in contact with steam and heat. Chemical indicators, e.g. autoclave tape, must be used with each load placed in the autoclave. The use of autoclave tape alone is not an adequate monitor of efficacy. Autoclave biological sterility monitoring should be conducted on a regular basis using appropriate indicators (*B. stearothermophilus* spore strips) placed at locations throughout the autoclave. The spores, which can survive 250° F for 5 minutes but are killed at 250° F in 13 minutes, are more resistant to heat than most, thereby providing an adequate safety margin when validating decontamination procedures. Each type of container employed should be spore tested because efficacy varies with the load, fluid volume, etc.
2. **Dry heat** is less efficient than wet heat and requires longer times and/or higher temperatures to achieve sterilization. It is suitable for the destruction of viable organisms on impermeable non-organic surfaces such as glass, but it is not reliable in the presence of shallow layers of organic or inorganic materials which may act as insulation. Sterilization of glassware by dry heat can usually be accomplished at 160-170° C for periods of 2-4 hours. Dry heat sterilizers should be monitored on a regular basis using appropriate biological indicators [ *B. subtilis*(*globigii*) spore strips].
3. **Incineration** is another effective means of decontamination by heat. As a disposal method incineration has the advantage of reducing the volume of the material prior to its final disposal.

### b. Liquid disinfection

The most practical use of liquid disinfectants is for surface decontamination and, when used in sufficient concentration, as a decontaminant for liquid wastes prior to final disposal in the sanitary sewer. If liquid disinfectants are used, they must have been shown to be effective against the organism(s) present.

Liquid disinfectants are available under a wide variety of trade names. In general, these can be classified as: halogens, acids, alkalis, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols and amines. The more active a compound is, the more likely it is to have undesirable characteristics such as corrosivity. No liquid disinfectant is equally useful or effective under all conditions and for all viable agents. Properties of common disinfectants may be found in Appendix D.

### c. Vapors and Gases

A variety of vapors and gases possess decontamination properties. Vapors and gases are primarily used to decontaminate biological safety cabinets and associated systems, bulky or stationary equipment not suited to liquid disinfectants, instruments or optics which might be damaged by other decontamination methods, and rooms, buildings and associated air-handling systems. Agents included in this category are glutaraldehyde and formaldehyde vapor, ethylene oxide gas, peracetic acid and hydrogen peroxide vapor. When used in closed systems and under controlled conditions of temperature and humidity, excellent disinfection can be obtained. Great care must be taken during use because of the hazardous nature of many of these compounds. Contact OEHS for monitoring requirements if these compounds are to be used.

### d. Radiation

Although ionizing radiation will destroy microorganisms, it is not a practical tool for laboratory use. Nonionizing radiation in the form of ultraviolet radiation (UV) is used for inactivating viruses, bacteria and fungi. It will destroy airborne microorganisms and inactivate microorganisms on exposed surfaces or in the presence of products of unstable composition that cannot be treated by conventional means.

Because of the low penetrating power of UV, microorganisms inside dust or soil particles will be protected from its action, limiting its usefulness. UV is used in air locks, animal holding areas, ventilated cabinets and laboratory rooms to reduce levels of airborne microorganisms and maintain good air hygiene. Because UV can cause burns to the eyes and skin of people exposed even a short period of time, proper shielding should be maintained when it is in use. UV lamps for that are used for space decontamination should be interlocked with the general room or cabinet illumination, so that turning on the lights extinguishes the UV.

UV lamps are not recommended for decontamination unless they are properly maintained. Because UV lamp intensity or destructive power decreases with time, it should be checked with a UV meter yearly. Frequent cleaning every few weeks is necessary to prevent accumulation of dust and dirt on the lamp which also reduces its effectiveness drastically. If UV must be used, it should be used when areas are not occupied.

## E. Infectious Waste Management

1. Categories of infectious waste as defined by the Commonwealth of Massachusetts, Department of Public Health include:

**a. Blood and blood products:** discarded bulk human blood products in free draining, liquid state; body fluids contaminated with visible blood; and materials saturated/dripping with blood.

**b. Pathological waste:** human anatomical parts, organs, tissues, and body fluids removed and discarded during surgery or autopsy, or other medical procedures and specimens of body fluids and their containers.

**c. Culture and stocks of infectious agents and associated biologicals:** all discarded cultures and stocks of infectious agents and associated biologicals, biotechnological by-product effluents (any discarded preparations made from genetically altered living organisms and their products), cultures of specimens from medical and pathological laboratories, cultures and stocks of infectious agents from research laboratories, wastes from the production of biologicals, and discarded live attenuated vaccines intended for human use.

**d. Contaminated animal carcasses, body parts and bedding:** the contaminated carcasses and body parts and bedding of all research animals known to be exposed to pathogens.

**e. Sharps:** discarded medical articles that may cause puncture or cuts, including but not limited to all used and discarded hypodermic needles and syringes, pasteur pipettes, broken medical glassware, scalpel blades, disposable razors, and suture needles.

## 2. Handling

All infectious waste from University laboratories must be autoclaved by the generator prior to disposal in appropriate infectious waste bags with labels. Treatment of infectious waste, other than by autoclaving, must be reviewed by the Office of Environmental Health and Safety.

The primary responsibility for identifying and disposing of infectious material rests with principal investigators or laboratory supervisors. This responsibility cannot be shifted to inexperienced or untrained personnel.

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

a. Used Sharps

b. Autoclavable Material

c. Non-Autoclavable Material for Incineration

**Used sharps** must be segregated into sharps containers that are nonbreakable, leakproof, impervious to moisture, rigid, tightly lidded, puncture resistant, red in color and marked with the universal biohazard symbol.

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

Other infectious waste must be discarded directly into containers or plastic (polypropylene) autoclave bags which are clearly identifiable and distinguishable from general waste. Containers must be marked with the universal biohazard symbol (Figure 1). Autoclave bags must be distinctly colored red or orange, and marked with the universal biohazard symbol. These bags must not be used for any other materials or purpose.

Decontaminated infectious waste must be put into black plastic bags and labeled appropriately. Infectious waste must be properly packaged prior to off site transport for destruction and disposal.

For specific information on infectious waste disposal procedures and pickup locations in your facility, call OEHS. Any off-site treatment of infectious waste must be coordinated through OEHS (373-2769).

### 3. Mixed Waste

Provisions must be made for potentially infectious waste with multiple hazards, e.g., radioactive material contaminated wastes, or wastes substantially contaminated with toxic/carcinogenic compounds. Contact OEHS regarding the disposal of these wastes.

### 4. Storage

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

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

If infectious waste becomes putrescent during storage it must be moved off site within 24 hours for processing and disposal.

Sharps containers may be used until 3/4 full, at which time they must be disposed of as infectious waste.

## 5. Monitoring Treatment of Infectious Waste

Autoclaving of infectious waste should be monitored to assure the efficacy of the treatment method (See P. 16). A log noting the date, test conditions and the results of each test of the autoclave must be kept.

## 6. Animals

University Laboratory Animal Resources must approve the disposal of research animals and animal parts that are considered to be infectious waste.

## F. Packaging and Shipping of Biomedical Materials

Etiologic agents, infectious materials and vectors that may contain them are recognized by the federal government and state government as hazardous materials. Infectious materials are regularly transported from one location to another by common land and air carriers. Containers of infectious materials must be carefully packaged to prevent leakage or breakage and consequent exposure to package contents. Packaging instructions are provided below.

Packaging and shipping of biomedical material must meet federal requirements. Regulations governing the interstate shipment of etiologic agents are currently under revision. The shipper (i.e., person with direct knowledge of what is being shipped) must be acquainted with the most current requirements. It is the intent of the regulation that biomedical material which may contain etiologic agents will be packaged and shipped in such a way that the contents will not leak and will arrive in good condition. The following definitions apply:

**Biomedical materials** that are known to contain or could contain, etiologic agents are divided into two groups: “diagnostic specimens and biological products” and “materials containing certain etiologic agents”.

**Etiologic agents** are those viable microorganisms that cause disease in humans and include bacteria, bacterial toxins, viruses, fungi, rickettsia, protozoans and parasites. These disease-causing microorganisms may also be referred to as infectious agents or infectious substances.

**Infectious substances** are those substances containing viable microorganisms or their toxins which are known, or are suspected to cause disease in animals or humans.

**Diagnostic specimens** are any human or animal material including but not limited to, excreta, secretions, blood and its components, tissue, tissue fluids, etc., which the shipper reasonably believes may contain an etiologic agent and that is being shipped for purposes of diagnosis.

**Biological product** means a product prepared in accordance with regulations that govern the manufacture of vaccines, reagents, etc.

**Materials containing certain etiologic agents** means materials known to contain or reasonably believed by the shipper to contain an etiologic agent from a list included in the regulation. The list contains most of the Class 2, 3 and 4 agents but any etiologic agent should be handled according to the regulation even if it is not on the list. Patient specimens that are expected to contain an etiologic agent should be shipped according to these requirements.

**Interstate shipping** is interpreted to include intrastate shipping.

**Packaging of diagnostic specimens and biological products** should be such that the package will withstand leakage of contents, shocks, pressure changes and other conditions incident to ordinary handling in transportation. Contents should not leak to the outside of the shipping container, even if leakage of the primary container occurs. Packages should be able to withstand rough handling and passage through cancellation machines, sorters, conveyers, etc.

**Packaging of materials containing etiologic agents** varies depending on the volume shipped.

**For volumes not exceeding 50 ml:**

The material to be shipped must be placed in a securely closed, watertight primary container. The primary container must be placed in a durable, watertight secondary container. Several primary containers may be placed in a single secondary container, so long as the total contents does not exceed 50 ml. Absorbent material must be placed in the spaces between the primary and secondary containers, so that there is enough absorbent to absorb the entire contents of the primary container(s) should breakage or leakage occur. Each set of primary and secondary containers must be placed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood or other material of equivalent strength. (Most bags and envelopes are not acceptable). See Figure 2 on the following page.

**For volumes greater than 50 ml:**

Packaging of these larger volumes must comply with the above-mentioned requirements. In addition, shock absorbent material in volume at least equal to that of the absorbent material must be placed between the secondary container and the outer shipping container. Single primary containers must not contain more than one liter of material. However, two or more primary containers, whose volumes do not exceed one liter may be placed in a single secondary container. The maximum amount of etiologic agent that may be enclosed within a single outer shipping container may not exceed four liters.



If dry ice is used, it must be placed between the secondary container(s) and the outer shipping container and the shock absorbent material placed so that the secondary container(s) do not become loose within the outer shipping container as the dry ice sublimates.

A special label, illustrated in Figure 2, must be placed on the outer shipping container. This label identifies the package as containing etiologic agents and directs anyone observing damage to the package or leakage of its contents to call CDC.

Certain etiologic agents require special handling in addition to that stated above. Most of these agents are in Class 3 and Class 4. They must be shipped by registered mail or an equivalent system which requires or provides for sending notification of receipt to the sender immediately upon delivery. When this notice of receipt is not received within 5 days following anticipated delivery the sender must notify CDC.

Questions pertaining to proper shipping and packaging of etiologic agents should be directed to OEHS at 373-2769 or the Centers for Disease Control and Prevention, Office of Health and Safety at (404) 639-3883.

*For shipments made internationally or domestically by carriers such as FEDEX, the International Air Transport Association (IATA) Dangerous Goods Regulations must be followed. Appropriate labels must be applied to the outer shipping container for packages that contain dry ice and/or infectious substances as shown in Figure 3 and Figure 4 respectively. More information on additional packaging and labeling requirements may be obtained by contacting the specific carrier's dangerous goods agent prior to shipment.*

## **G. Importation/Exportation of Etiologic Agents**

Importation of infectious materials, etiologic agents and vectors that may contain them is governed by federal regulation. In general, an import permit is required for any infectious agent known to cause disease in man. This includes but is not limited to bacteria, viruses, rickettsia, parasites, yeasts and molds. In some instances, an agent which is suspected of causing human disease also requires a permit.

The following vectors require import permits:

- Animals known or suspected of being infected with any disease transmissible to man. Importation of turtles less than 4 inches in shell length and all nonhuman primates requires an importation permit issued by the United States Public Health Service (USPHS) Division of Quarantine, (404)639-1437.
- Biological materials: Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions or similar material, when known or suspected to be infected with disease transmissible to man.
- Insects: Any living insect or other living arthropod, known or suspected of being infected with any disease transmissible to man. Also, if alive, any fleas, flies, lice, mites, mosquitoes or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.
- Snails: Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either CDC or the Department of Agriculture (see below for phone numbers). Any shipment of mollusks with a permit from either agency will be cleared immediately.
- Bats: All live bats. Bats may also require a permit from the U. S. Department of the Interior, Fish and Wildlife Services (USDI; see below for phone number).

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

Shipping labels containing the universal biohazard symbol, the address of the importer, the permit number and the expiration date are issued to the importer with the permit. The importer must send the labels and one or more copies of the permit to the shipper. The permit and labels inform the U. S. Customs Service and the U.S. Division of Quarantine personnel of the package contents.

The importer bears responsibility for assuring that the foreign shipping personnel pack and label the infectious materials according to USPHS regulations. Transfers of previously imported material within the United States also require a permit.

Instead of an importation permit, a “Letter of Authorization” may be issued by the issuing officer after review of an “Application to Import an Etiological Agent”. The letter is issued for materials that are judged to be noninfectious, but which might be construed to be infectious by U. S. Customs inspection personnel. Letters of Authorization may be issued for items such as formalin fixed tissues, sterile cell cultures, clinical materials such as human blood, serum, plasma, urine cerebrospinal fluid, and other tissues or materials of human origin when there is no evidence or indication that such materials contain an infectious agent. Letters of Authorization are in effect for two years, and do not require a shipping label to be issued by CDC.

Etiologic agents, infectious materials and vectors that may contain them must be carefully packaged to prevent leakage or breakage and consequent exposure to the package contents. The package must be labeled with the universal biohazard sign to warn package handlers of the hazardous contents. (See packaging instructions above).

Importation permits and Letters of Authorization are issued by the Biosafety Branch, Office of Health and Safety, CDC, after review of a completed application form. Application forms may be obtained directly from OEHS (898-4453) or by calling CDC at (404)639-3883. Completed forms may be returned to CDC by mail or FAX. Application to CDC for the importation permit should be made 10 working days in advance of the shipment date to allow time for processing, issuance and delivery of the permit and shipping labels to the permittee.

### **Other permits:**

U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required for infectious agents of live stock and biological materials containing animal, particularly livestock, material. Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA

due to the potential risk of introduction of exotic animal disease into the U. S. Applications for USDA/APHIS permits may be obtained from OEHS (373-2769). Further information may be obtained by calling the USDA/APHIS at (301) 436-7885.

USDI permits are required for certain live animals and all live bats. Call (800)358-2104 for further information.

Export of infectious materials may require a license from the Department of Commerce. Call (202)482-0896 for further information.