Section 4 – Procedures for Biohazard Control

Contents

A. FACILITY REQUIREMENTS

1. BSL-1 Laboratory Facilities

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

2. BSL-2 Laboratory Facilities (in addition to BSL-1 requirements stated above)

1. Laboratory doors are locked when not occupied.
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. Chairs used in the laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate decontaminant. Fabric chairs are not allowed.
4. An eye wash station is readily available (within 50 feet of workspace and through no more than one door).
5. Ventilation - Planning of new facilities should consider ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. See the Laboratory Safety Design Guide for specifications.
6. 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.
7. Biological Safety Cabinets (BSCs) are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible sources of airflow disruptions.
8. Vacuum lines should be protected with in-line High Efficiency Particulate Air (HEPA) filters.
9. HEPA-filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system either by a thimble (canopy) connection or by exhausting to the outside directly through a hard connection. Proper BSC performance and air system operation must be verified at least annually.

3. BSL-2 with BSL-3 practices Laboratory Facilities (in addition to BSL-2 and BSL-1 requirements stated above)

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

4. BSL-3 Laboratory Facilities (in addition to BSL-2 requirements stated above)

1. Laboratory doors are self-closing and the outside door must be locked at all times. The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Hands-free or automatically operated sinks for hand washing are installed near exit door and in each laboratory.
3. Walls and ceiling surfaces are sealed and have a smooth finish.
4. Floors are slip resistant, impervious to liquids, and resistant to chemicals.
5. All windows in the laboratory are sealed.
6. Ventilation - A ducted air ventilation system provides directional airflow by drawing air into the laboratory from clean areas toward potentially contaminated areas. The laboratory is designed such that, under failure conditions, the airflow is not reversed. Laboratory personnel are able to verify directional air flow with a visual monitoring device. Laboratory exhaust air does not recirculate to any other area of the building.
7. At the UW, the laboratory building exhaust air is HEPA filtered and dispersed away from occupied areas and from building air intake locations. The filters and the housing are certified at least annually.
8. Decontamination - A method for decontaminating all lab wastes is available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated method).
9. Verification and Documentation - At the UW, the BSL-3 facility design, operational parameters, and procedures are verified and documented by an outside contractor prior to operation. Facilities are verified that they meet the intent of the current edition of the CDC/NIH *BMBL* and documented at least annually.

B. GOOD LABORATORY PRACTICES AND TECHNIQUES

1. Technical Proficiency

Laboratory personnel must be aware of the potential hazards and must be trained and proficient in the necessary practices and techniques required for safe handling of biohazardous agents. Laboratory personnel must have documented training in handling biohazardous

agents. The PI is responsible for providing or arranging for appropriate training for all personnel working in their laboratory.

Additional training for BSL-2 laboratories following BSL-3 practices and BSL-3 laboratories

Laboratory personnel working in BSL-2 laboratories with BSL-3 practices or in BSL-3 laboratories must have documented training on the laboratory-specific biosafety manual and practical training with the PI. All required EH&S safety classes (e.g., biosafety training, bloodborne pathogens training) must be current.

2. Hazard Awareness Training

a. Biosafety Training

Completion of the EH&S online Biosafety training is required every three years for PIs if their research includes the use of biohazardous agents. It is also required for students, fellows, laboratory managers, research staff, and all other staff who have the potential for exposure to recDNA and other biohazardous agents. This training is required before initiating research with biohazardous agents, including recDNA, and every three years thereafter.

b. Bloodborne Pathogens Training

Staff with reasonably anticipated potential for exposure to human blood, human source material, all human cell lines, and other potentially infectious materials must take either the in-person or online EH&S bloodborne pathogens (BBP) training. The training is required initially and every year thereafter. .

3. Prohibited Activities

a. Eating, drinking, handling contact lenses, applying cosmetics, chewing gum, and storing food for human consumption is not allowed in the work area of the laboratory. Smoking is not permitted in any University building. Food shall not be stored in laboratory refrigerators or prepared/consumed with laboratory glassware or utensils. Food may be stored in cabinets and refrigerators marked for "FOOD ONLY." These must be located outside the laboratory work area and physically separated by a door from the main laboratory.

b. Mouth pipetting is prohibited in research laboratories; only mechanical pipetting devices can be used.

c. Storage of laboratory equipment in public corridors - There are restrictions on storage of laboratory equipment in public corridors. Information on storage in hallways and stairwells is available online.

d. Animals and plants not associated with the work being performed are not permitted in the laboratory.

e. Personal protective equipment (e.g., lab coats, gloves) cannot be worn in public hallways.

f. Biohazardous agents (including biohazardous waste) cannot be transported in public corridors without a secondary container.

4. Personal Protective Equipment

Specific rules concerning personal and protective clothing must be devised by the PI. It is important to recognize that hair, beards, personal clothing, and shoes can effectively disseminate infection. (See fomites in Section 4.A.4).

a. Laboratory Coats

a. BSL-1 laboratories: Laboratory coats are recommended for general biological work in a BSL-1 laboratory and when working with BSL-1 biohazardous agents, including BSL-1 recDNA. Laboratory coats may also be necessary when working with chemicals, radioisotopes, etc.

b. BSL-2 laboratories: Dedicated laboratory coats, gowns, or smocks are worn while working in the BSL-2 laboratory area. Before moving from the BSL-2 laboratory area to a non-BSL-2 laboratory area (e.g., BSL-1 laboratory, hallway, cafeteria, library, administrative offices), protective clothing must be removed and left in the laboratory. Reusable laboratory coats must be laundered on a regular basis and are never to be taken home.

c. BSL-2 with 3 practices and BSL-3 practices laboratories: All the rules for BSL-2 laboratory apply. In addition, laboratory clothing that protects street clothing (solid front or wrap-around gowns, scrub suits or coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory; the clothing is autoclaved before laundering or disposal. Tight fitting cuffs on laboratory clothing or sleeve protectors are useful.

b. Gloves

Glove selection should be based on an appropriate risk assessment. In laboratory settings, the most common gloves are latex and nitrile. Both are appropriate for protection against biohazardous agents. However, these gloves are not intended to provide protection from punctures caused by sharp instruments or broken glass.

If work involves the use of chemicals with biohazardous agents, select gloves according to recommendations in the Laboratory Safety Manual, Appendix G and refer to the associated Material Safety Data Sheet (MSDS). Many chemicals destroy the integrity of latex gloves (e.g., do not use 70% ethanol with latex gloves).

1) Gloves must always be visually checked for defects before using (e.g., look at gloved hands).

2) Gloves should be changed when contaminated, torn, or punctured. Care should be taken not to touch your skin with the outer surface of the gloves when removing them. Wash hands immediately after gloves are removed and before leaving the laboratory.

3) Gloves are removed prior to handling non-contaminated items such as doorknobs or telephones. Gloves are not worn outside the laboratory area.

4) Do not wash or disinfect and then reuse disposable gloves. Detergents may cause enhanced penetration of liquids through undetected holes and disinfectants may cause deterioration.

5) Used gloves must be treated as biohazardous waste and decontaminated prior to disposal.

Double glove practices must be used in BSL-2 laboratories following BSL-3 practices and BSL-3 laboratories.

c. Facial Protection

Facial barrier protection is required for activities in which there is a potential for splash/splatter of biohazardous agents onto the mucous membranes of the mouth, nose, and eyes.

Eye and face protection - Goggles, mask, face shield, or other splatter guards are used for anticipated splashes or splatters of biohazardous agents when the agents 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 must also wear eye protection.

Face Shields - Full face shields made of lightweight transparent plastic are the preferred means of facial protection. They offer excellent protection of the entire face and neck region. They are easily decontaminated. Face shields can also be used with a mask or respirator. If face shields are not used, a combination of mask and eye protection should be used whenever splashes, spray, or splatter of biohazardous agents may be generated and where eyes, nose, or mouth contamination can be reasonably anticipated.

Surgical Masks with liquid barriers - A surgical mask offers protection of the nose and mouth. Either soft or preformed masks are effective. Surgical masks protect the mucous membranes in the mouth and nose from splashes or splatters. Surgical masks do not protect against aerosols.

Goggles/Safety Glasses with side shields - Ordinary prescription glasses are not adequate eye protection. Plastic safety glasses with side shields that fit over regular glasses should be used. If there is a substantial hazard for splattering, safety goggles with a plastic cushion seal should be used. Goggles, which seal around your eyes, are preferred over safety glasses with side shields.

Respirators - A respirator protects the mouth and the respiratory tract from aerosols. Based on EH&S risk assessment, a respirator may be needed if aerosols are generated outside of appropriate containment.

Handling Infected Animals - Eye, face, and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Molded surgical masks or respirators are worn in ABSL-3 rooms containing infected animals. Gloves are worn when handling infected animals and when there is potential skin contact with biohazardous agents.

Alternatives of facial barrier protection:

1) Use a BSC **-** Perform the manipulations in a Class II BSC.

2) Use a splash shield **-** Purchase or construct a splash shield that can be placed on the bench top to provide a physical barrier. A clear plastic shield provides an effective barrier for potential splashes from opening tubes. It is not effective for manipulations that create major aerosols. Such manipulations must be performed in a BSC.

5. Restrict Traffic in Laboratories

Traffic should be restricted in a BSL-1 laboratory. How that is enforced is at the discretion of the PI. However, this requirement must be enforced rigorously with respect to biohazards and recDNA in BSL-2, BSL-2 laboratories with BSL-3 practices, and BSL-3 laboratories. There is no research requiring BSL-4 containment at the UW.

a. Access to a BSL-2 laboratory is restricted - The door to the laboratory is closed and the BSL-2 Biohazard Warning Sign is displayed when use of biohazardous agents is in progress. The door to the laboratory is locked when unoccupied.

b. Access to BSL-2 laboratories and BSL-2 laboratories with BSL-3 practices is restricted - The door to the laboratory remains locked at all times and the BSL-2 with BSL-3 practices Biohazard Warning Sign is permanently affixed to the door.

c. Entry to BSL-3 laboratories is restricted by a double set of doors. The outer door of the BSL-3 laboratory is locked at all times and the Biohazard Warning Sign is permanently affixed to the door.

6. Biohazard Warning Door Sign

The sign must include the name of the agent(s) in use, and the name and phone number of the PI or other responsible personnel. See Appendix B for additional information on the use of the Biohazard Warning Sign.

7. Handwashing

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

8. Biosafety Manual and Laboratory-Specific Biosafety Manual

BSL-1 and BSL-2 laboratories must have access to a current copy of the UW Biosafety Manual by a prominently displayed icon on a computer desktop or a hard copy. BSL-2 laboratories with BSL-3 practices and BSL-3 laboratories must also have a laboratory specific biosafety manual with written standardized safety procedures that have been reviewed by an EH&S BSO.

9. Good Housekeeping

Work areas must be free of clutter and cleaned regularly. Wet mopping is the preferred method over dry sweeping or the use of ordinary vacuums which create aerosols.

Work surfaces are decontaminated once a day and after any spill of potentially viable material. Decontamination is covered in Section 4.E and spill cleanup is in Section 6.A of this manual.

10. Inventory Control

Laboratories should have a process for controlling inventory of infectious agents. All microorganisms stored in the lab should be documented and labeled. Any stocks or cultures that are not needed should be decontaminated and disposed of properly. If any select agents or select toxins are discovered, contact EH&S immediately for assistance.

11. Pest Control

Pest control is best accomplished by maintaining good housekeeping. A good sanitation program is fundamental to the control of vermin and should include a program of storage, collection, and disposal of solid wastes. Caulking of cracks and crevices in the room is also important.

The UW employs a licensed pest control operator to control vermin in strict accordance with applicable laws and regulations. Contact EH&S (206-543-7209) if vermin problems are suspected so that a control program can be implemented.

12. Biohazardous Waste

All biohazardous liquid or solid wastes are decontaminated before disposal. This includes waste from research with all forms of recDNA. Sharps containers must not be filled to more than two-thirds full. Decontamination is covered in Section 4.E and waste disposal is covered in Section 4.F.

13. Minimization of Aerosols

All procedures are performed carefully to minimize the creation of aerosols. Use BSCs or other physical containment devices whenever aerosol generating procedures at BSL-2 are conducted (e.g., pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, or opening containers of biohazardous agents).

In BSL-2 laboratories with BSL-3 practices and BSL-3 laboratories, open manipulations of all biohazardous agents must be conducted inside a BSC or other physical containment device.

C. LABORATORY EQUIPMENT AND PROCEDURES

This section describes the different types and proper use of laboratory safety equipment (e.g., BSCs, blenders, ultrasonic disintegrators, grinders, mortar and pestle, automated equipment, water baths, incubators, refrigerators, deep freeze, dry ice chests, vacuum lines, and microtome/cryostat).

This section further describes proper techniques used when working with biohazardous agents (e.g., pipetting; working outside a BSC; using syringes and needles; opening culture plates, test tubes, bottles, or ampoules; handling laboratory glassware; cell sorting; and centrifugation).

1. Laboratory Equipment

Equipment must be marked with the biohazard symbol or the word biohazard where it is necessary to alert personnel of the potential for exposure. Refer to Appendix B for additional labeling information.

Equipment which may be contaminated with blood or potentially infectious materials must be decontaminated prior to servicing. When a portion of the equipment cannot be decontaminated, the equipment must be labeled with the biohazard label and a sign stating which portion of the equipment remains contaminated. This information must be conveyed to all repair workers and servicing representatives and/or the manufacturer as necessary prior to handling, servicing, or shipping so that appropriate precautions can be taken.

Equipment being repaired, surplused, or disposed of must be decontaminated. A Notice of Laboratory Equipment Decontamination (Form UoW 1803) must be completed to certify decontamination.

The proper use of some commonly used laboratory equipment is described below.

a. Biological Safety Cabinet (BSC)

The BSC is designed to reduce the potential escape of research material into the worker's environment and to remove contaminants from the research work zone.

The following types of Class II BSCs provide a clean work zone (product protection), aerosol protection for the operator (personnel protection), and environmental protection through use of a HEPA filter. HEPA filters are effective at trapping particulates and thus infectious agents. They do not capture volatile chemicals or gases. Only Type A2 exhausted or Types B1 and B2 BSCs exhausting to the outside should be used when working with volatile toxic chemicals. In any case, amounts of these chemicals must be limited.

1) Equipment to protect the worker, product, and environment

Class II, Type A1 BSCs are suitable for work with low to moderate risk biological agents requiring BSL-1, BSL-2, or BSL-3 containment in the absence of volatile toxic chemicals and volatile radionuclides. The buildup of chemical vapors in the cabinet

(by recirculated air) and in the laboratory (from exhaust air) could create health and safety hazards.

Class II, Type A2 BSCs (formerly designated Type B-3) are suitable for work with low to moderate risk biological agents requiring BSL-1, BSL-2, or BSL-3 containment. Minute quantities of volatile toxic chemicals or volatile radionuclides can be used in a Type A2 cabinet only if the cabinet exhausts to the outside via a properly functioning canopy connection.

Class II, Type B1 BSCs are suitable for work with biological agents requiring BSL-1, BSL-2, or BSL-3 containment. They may also be used with biological agents treated with toxic chemicals and trace amounts of radionuclides required as an adjunct to microbiological studies if work is done in the direct exhausted portion of the cabinet or if the chemicals or radionuclides will not interfere with work when recirculated in the downflow.

Class II, Type B2 BSCs are suitable for work with biological agents requiring BSL-1, BSL-2, or BSL-3 containment. They may also be used with biological agents treated with toxic chemicals and radionuclides required as an adjunct to microbiological studies. This type of cabinet is sometimes referred to as a "Total Exhaust Cabinet."

2) Equipment that can be used to provide limited personnel protection but no product protection

   A **Class I** BSC is similar to a fume hood in its basic design and personnel protection capabilities. This cabinet can be used for work at BSL-2 containment when minimal personnel protection and no product protection is required. The cabinet's exhaust air is filtered through a HEPA filter. The filter provides a significant degree of environmental protection, which a fume hood does not offer.

3) Equipment that can be used to provide limited product protection and no personnel protection

   Non-ventilated tissue culture boxes provide an air circulation free enclosure for sterile techniques. It provides no personnel protection and some product protection. Its use is limited to BSL-1 laboratories.

   Horizontal laminar flow units provide a work area free of contaminants. The HEPA filtered air blows directly onto the operator so no personnel protection is provided. The use of this type of unit is limited to the preparation of sterile media, assembly of sterile components into complete units, the examination of sterilized equipment and materials for possible contamination, and other similar operations. Work with live agents is not permitted. The equipment must be labeled "NOT for Use with Pathogenic Organisms."

   Vertical laminar flow units provide a work area free of contaminants. The HEPA filtered air does not blow directly on to the operator but is exhausted either from the top or bottom of the unit. The use of this type of unit is limited to the preparation of sterile media, assembly of sterile components into complete units, the examination of sterilized equipment and materials for possible contamination, and other similar operations. The equipment must be labeled "NOT for Use with Pathogenic Organisms."

4) BSC certification

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

spills. Decontamination can be done using paraformaldehyde. Contact EH&S at 206-543-9510 for information on decontamination.

The methods and requirements for testing BSCs vary depending upon the design of the cabinet and its intended use. While structural certification of the BSC is made by the manufacturer prior to shipment, stress during shipment can alter the integrity and efficiency of the BSC.

All research materials must be removed from the BSC prior to testing and certification. Plan and schedule in advance as the BSC cannot be used until certification is complete.

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

The testing and certification process includes:

- A leak test to assure that the airflow plenums are gas tight in certain installations.
- A HEPA filter leak test to assure that the filter, the filter frame, and filter gaskets are all properly in place and free from leaks. A properly tested HEPA filter will provide a minimum efficiency of 99.99% on particles 0.3 microns in diameter and larger.
- Measurement of airflow to assure that velocity is uniform and unidirectional.
- Measurement and balance of intake and exhaust air.

Users must receive training prior to use of BSCs. This training is the responsibility of the PI.

5) Basic guidelines for working in a BSC

- Never place anything over the intake or rear exhaust grill. Keep equipment at least four inches inside the cabinet window and perform all transfer operations of viable material as deeply into the BSC as possible.
- Do not overload BSC with equipment and other items. Only bring in items needed for work.
- Plan in advance to have all required equipment inside the BSC. Good laboratory technique minimizes arm movements through the air barrier until the procedure is completed.
- During manipulations inside the BSC, segregate contaminated and clean items. Keep clean items out of the work area, and place discard containers to the rear of the BSC.
- Avoid entrance and exit from the workroom. Foot traffic can cause disruptive drafts that allow microorganisms to escape through the air barrier of the BSC.
- Equipment should be kept as parallel as possible to the downflow of the airstream.
- To purge airborne contaminants from the work area, allow the BSC to run following completion of work. The BSC can be turned off after 20 minutes but it is recommended that it be left on continuously.
- Decontaminate the BSC after use (see Section 4.E).

- Do not use an open flame Bunsen burner inside a BSC. If required, a touch-amatic burner or infrared loop sterilizer should be used. An open flame Bunsen burner disrupts the unidirectional air stream. The flame could damage the filter or set fire to the BSC when the BSC is turned off.
- Do not use the BSC for storage when not in use.

b. Blenders, ultrasonic disintegrators, grinders, mortar, and pestle

All of these devices release considerable aerosols during their operation. For maximum protection to the operator during the blending of biohazards, the following practices should be observed:

1) Operate blending, cell-disruption, and grinding equipment in a BSC.

   Or

2) Use a heat-sealed flexible plastic film enclosure for a grinder or blender. The grinder or blender must be opened in a BSC.

c. Automated equipment

Clinical or other laboratory personnel handling human blood, non-human primate blood, and other biohazards should be aware of aerosols produced by the micro-hematocrit centrifuge, the autoanalyzer, and the microtonometer.

d. Water baths and incubators

After use, water baths and incubators must be decontaminated with an appropriate decontaminant (see Section 4.E).

Maintenance service on water baths and incubators that appear to be improperly used and/or contaminated may be denied. It is not the responsibility of maintenance personnel to clean up after laboratory personnel.

e. Refrigerators, deep freeze, and dry ice chests

Deep freezers, liquid nitrogen, dry ice chests, and refrigerators should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing biohazards.

Containers must be stored in proper order and sequence and properly labeled to preclude withdrawal of the wrong ampoules or tubes. Use of gloves and respiratory protection during cleaning of refrigerators, deep freeze or dry ice chests is recommended. All materials that are stored should be properly labeled with the scientific name, the date stored, and the name of the individual storing the material.

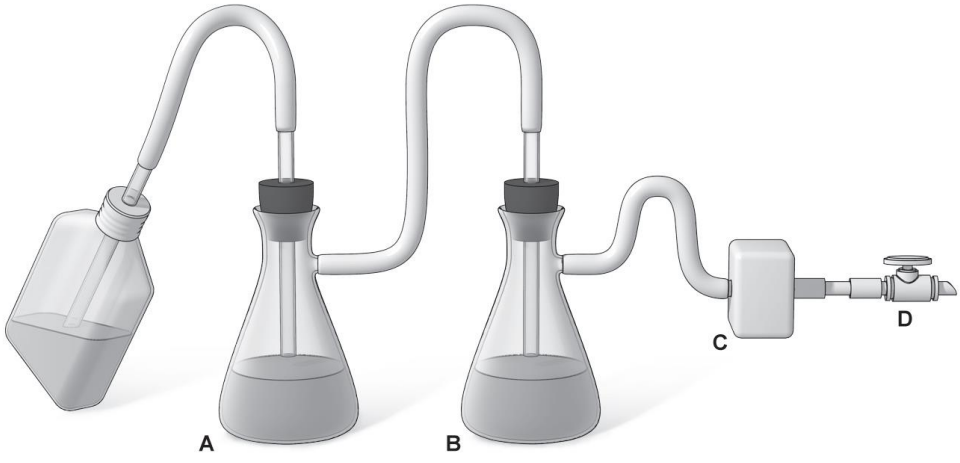
Flammable solutions that require 4 degree storage conditions must be stored in a refrigerator approved for flammable storage. Contact EH&S at 206-543-2835 or visit the EH&S page for additional information on flammable storage.

f. Laboratory vacuum lines

Appropriate in-line safety reservoirs and filters ensure that laboratory vacuum lines do not become contaminated with biohazardous agents. Aspirator bottles or suction flasks (Figure 1, A) should be connected to an overflow collection flask (Figure 1, B) containing appropriate disinfectant and to an in-line HEPA or equivalent filter (Figure 1, C). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution (e.g., bleach) into the flask to inactivate the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of in the sink. In-line HEPA filters

must be replaced as needed. If glass flasks are used, they should be placed in leak-proof secondary containment in the event of a break or spill.

Figure 1 Protecting Laboratory Vacuum Line



One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

g. Using a Microtome/Cryostat

The microtome and the cryostat are used for cutting thin sections of fixed and unfixed tissue. The use of microtomes and cryostats in the laboratory presents a laceration hazard in addition to generating potentially infectious aerosols. Unfixed tissues should be considered capable of causing infection and should be treated with care. Employees who handle or could be exposed to tissue of human origin must be enrolled in the UW BBP Program (Appendix A).

Observe the following procedures when using microtomes/cryostats:

1) Always keep hands away from blades.
2) Position the sample first and then put in the blade with the blade edge positioned away from hands.
3) Use engineering controls like forceps, tweezers, dissecting probes, and small brushes to retrieve samples, change blades, dislodge blocks, or clean equipment.
4) Use protectors/guards for knife-edges that may extend beyond the microtome knife holder.
5) Wear appropriate personal protective equipment (PPE) such as gloves, lab coat or gown, mask, and safety glasses or goggles. Consider the use of surgical grade Kevlar gloves when using a cryostat to provide additional protection from cuts and scrapes.
6) Do not leave motorized microtomes running unattended.
7) Discard and handle trimmings and sections of tissue as biohazardous waste.

8) Do not move or transport a microtome with the knife in position.

9) Always lock the chuck rotating mechanism (wheel) to immobilize the block when not actively cutting tissue and before insertion or removal of the blade.

10) Never walk away from an exposed blade.

11) At the end of each session with the microtome or cryostat, either dispose of the blade immediately in a sharps container or secure reusable blades in a container.

2. Laboratory Procedures

a. Pipetting

Delivery with the tip of the pipette resting against the container allows the fluid to flow down the surface and minimizes aerosols.

Allowing a droplet to fall from the tip of a pipette, intentionally or accidentally, results in aerosol production, the extent of which depends on the height of the fall and the surface upon which the droplet lands. The following procedures should be followed for pipetting:

1) Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.

2) Infectious mixtures should not be prepared by bubbling air through the liquid with the pipette.

3) Infectious materials should not be forcibly discharged from pipettes (e.g., the last drop forcefully removed).

4) A towel wetted with disinfectant or a soft absorbent pad covering the immediate work surface is most useful in absorbing droplets and small spills.

b. Working Outside a BSC Using a Splash Guard and/or Additional PPE

In cases where the biohazardous agent is not transmitted via a route of inhalation (e.g., opening tubes containing blood or body fluids), it is permissible to work outside a BSC using a splash guard. A splash guard is an example of a barrier type engineering control that protects by providing a shield between the user and any activity that could cause an aerosol or splatter. An example of such a splash guard is a simple clear plastic panel formed to stand on its own and provide a barrier between the user and activities such as opening tubes that contain blood or other potentially infectious materials (OPIMs). Additional PPE (e.g., safety goggles, glasses, face shield) may be required for splash protection when working with biohazardous materials outside a BSC.

c. Using Syringes and Needles

Extreme caution should be used to avoid accidental injection and the generation of aerosols during use and disposal. Use syringes and needles only for injection and aspiration of fluid from laboratory animals and diaphragm bottles.

1) Do not use a syringe and needle as a substitute for a pipette when making dilutions of fluids. Syringe type pipettes with blunt ended delivery are permissible.

2) Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe.

3) Prior to beginning an animal inoculation, be sure the animal is properly restrained. Swab the site of the injection with a suitable disinfectant. Inoculate the animal with a hand behind the needle to avoid punctures. Swab the injection site again with a suitable disinfectant.

4) Following use, needles should not be bent, sheared, replaced in the sheath or guard (capped), or removed from the syringe. The needle and syringe unit should promptly be placed in a leak-proof, rigid, puncture, and break resistant sharps container. The container is red in color and equipped with a tight fitting lid for use during handling and transport. The container must be decontaminated by autoclaving before discarding. Additional information on waste disposal is found in Section 4.F.

d. Opening Culture Plates, Tubes, Bottles, and Ampoules

Aerosol formation is the primary concern when plugs or screw caps are removed from tubes and bottles. Slow and smooth manipulations will minimize aerosols. See Section 4.B.4.c Facial Protection for additional information.

Opening ampoules is potentially hazardous since, after the seal is broken, the air rushes in causing the dry contents to be dispersed. A BSC should be used. The bottom of the ampoule should be held in several layers of lab wipes to protect the hands. Nick the neck of the ampoule with a file. A hot glass rod should be carefully applied to the mark. The glass will crack, allowing air to enter the ampoule and equalize the pressure. After a few seconds the ampoule should be wrapped in a few layers of lab wipes and broken along the crack.

An alternative method of opening an ampoule involves wearing gloves and other PPE, nicking the ampoule with a file, and wrapping the ampoule in disinfectant-wetted cotton for breaking.

In both methods the ampoule neck and other waste is handled as biohazardous sharps waste. Additional information on Waste Disposal is found in Section 4.F.

e. Using Test Tubes and Other Laboratory Glassware

Tubes containing biohazards should be manipulated with extreme care. Studies have shown that simple procedures such as removing the tube cap or transferring an inoculant can create a potentially hazardous aerosol. Tubes and racks of tubes containing biohazards should be clearly marked with agent identification.

Safety test tube trays should be used in place of conventional test tube racks to minimize spillage from broken tubes. A safety test tube tray is one that has a solid bottom and sides that are deep enough to hold all liquids if a tube should break.

Glassware breakage is a major risk for puncture infections. It is most important to use non-breakable containers where possible and carefully handle the material. Avoid unnecessary use of glass Pasteur pipettes. Whenever possible, use flexible plastic pipettes or other alternatives.

It is the responsibility of the PI and/or laboratory manager to assure that all glassware/plasticware is properly decontaminated prior to washing or disposal. See Section 4.E for additional information on decontamination.

For disposal recommendations see Section 4.F.

f. Cell Sorting

Clinical or other laboratories handling human blood, non-human primate blood, recDNA, and other biohazardous agents should be aware of aerosols produced by the cell sorter. High-speed cell sorting can produce aerosols that may present a health hazard to workers. To help ensure the safety of staff, the following additional safety measures are used:

1) Engineering controls, that may include an Aerosol Management System (AMS) that rapidly evacuates and filters aerosolized particles from the cell sorter chamber.

2) Safe practices, that may include additional PPE and training, are unique to each UW facility and must be followed as specified in the cell sorter core facility Manual of Standard Operating Procedures (SOPs).

3) EH&S and IBC approval may be required prior to sorting specific cell types, (e.g., all human cells including human cell lines, cells containing recDNA, cells exposed to virus or bacteria). Contact EH&S ROS for more information, ehsbio@uw.edu, 206-221-7770.

g. Centrifugation

Accidents resulting from the improper use of centrifuges and associated equipment occur less frequently than from the use of pipettes, syringes, and needles. However, if accidents do occur, aerosols are created and the possibility of causing multiple infections is considerably greater.

A mechanical failure (broken drive shaft, a faulty bearing, or a disintegrated rotor) can produce not only aerosols, but also hazardous fragments at great velocity. These fragments, if they escape the protective bowl of the centrifuge, could produce traumatic injury to personnel. Risk of mechanical failure can be minimized by meticulous observance of the manufacturer's instructions.

Even a well-functioning centrifuge is capable of producing biohazardous aerosols. However, aerosols can be avoided by observing sound laboratory practices and using appropriate centrifuge safety equipment and containment cabinets as described below.

Centrifugation of biohazardous agents including recDNA should be:

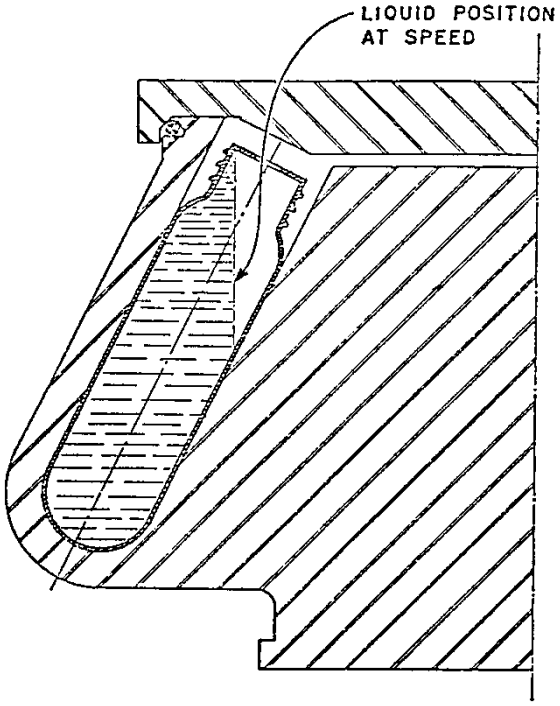
1) Performed in a centrifuge that is contained within a BSC

   or

2) If no such centrifuge is available, an aerosol containment device must be used. Aerosol containment devices include centrifuge sealed rotor heads or sealed safety cups.

Activities such as filling centrifuge tubes, removing cotton plugs and rubber caps on tubes after centrifugation, removing the supernatant, and resuspending the pellet can release aerosols into the environment. Centrifuge tubes and bottles should be filled and opened in a BSC. Do not fill tubes to the point that the rim of the closure becomes wet with culture. Special attention needs to be given when filling tubes to be placed in a fixed angle centrifuge.

Figure 2 Filling Centrifuge Tubes



Screw caps, or other tight-fitting skirted caps that fit outside the rim of the centrifuge tube, are safer to use than plug-in closures. Some fluid usually collects between a plug-in closure and the rim of the tube. Even screw capped bottles are not without risk; if the rim is soiled and seals imperfectly, some fluid will escape down the outside of the tubes. Aluminum foil should never be used to cap centrifuge tubes containing toxic or biohazards because these light-weight caps often become detached or ruptured during handling and centrifuging. When centrifuging biohazards, including clinical specimens, do not use cotton plugs. Instead, use tight-fitting tabbed or hinged caps made of plastic or rubber, screw caps, or other tight-fitting plastic or metal closures.

The aerosol containment device must be removed from the centrifuge and opened in the BSC. These devices often have clear tops to alert the operator to problems such as broken or leaking tubes prior to opening.

The greatest hazard associated with centrifuging biohazards is created when a centrifuge tube breaks. Avoid use of glass centrifuge tubes. Plastic tubes and bottles are a better option than glass centrifuge tubes because they resist breakage. However, they are not indestructible. Plastic containers may begin to show signs of deterioration after several runs as a result of the interaction of centrifugal forces, chemical effects from samples and cleaning solutions, and autoclaving cycles of heat and pressure. Deterioration may appear as crazing, cracking, or spotting. Tubes showing these signs should be used only at low speeds, used as storage containers, or discarded. Some plastics are subject to chemical interaction with samples being processed. For complete specific information, the PI/lab manager should refer to the material compatibility data provided by the manufacturers of the centrifuge equipment.

Proper balancing of the centrifuge is important. Care must be taken to ensure that matched sets of safety devices and adapters do not become mixed. If the components are not inscribed with their weights by the manufacturer, colored stains can be applied for identification to avoid confusion. The basic concern is that the center of gravity of the

tubes is equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20 g of mercury and 20 g of water, respectively, will balance perfectly on the scales; however, their performance in motion is totally different, leading to violent vibration with all its attendant hazards.

Cleaning and disinfection of tubes, aerosol containment devices, rotors, and other components require considerable care. It is unfortunate that no single process is suitable for all items. The various manufacturers' recommendations must be followed meticulously if fatigue, distortion, and corrosion are to be avoided. All components, including the sealing gaskets, must be inspected periodically for wear. When problems are noted, the components must be replaced.

In the event of a centrifuge malfunction and/or spill that may create hazardous aerosols, the room should be vacated by all personnel for a suitable period to allow the aerosol to dissipate (at least 30 minutes). Contaminated areas, broken glass, etc. should then be properly decontaminated and cleaned up promptly. The person using the centrifuge, along with the PI and/or laboratory manager, are responsible for ensuring that clean-up and decontamination is achieved.

Maintenance service may be refused on centrifuges which appear to be improperly used and/or contaminated. It is not the responsibility of maintenance personnel to clean up after laboratory personnel.

h. Resuspending Sediment of Centrifuged Material

Use a swirling, rotary motion, rather than shaking, to resuspend the sediment of packed biohazardous materials. This motion is preferred in order to minimize the amount of aerosol created. When performing these operations, a BSC may be required to assure the safety of the laboratory worker. If vigorous shaking is essential to suspend the material or achieve homogeneity, a few minutes should elapse before opening the container to allow the aerosol to settle. Shaking always contaminates the closure and there is the added hazard of liquid escaping and running down the outside of the container or dropping from the closure when it is removed.

D. CONTROL OF BIOHAZARDS ASSOCIATED WITH LABORATORY ANIMALS

1. Responsibility

Procedures designed to prevent exposure to or transmission of biohazards from laboratory animals to human beings must be taken into account. Both naturally occurring diseases of laboratory animals transmissible to humans and experimentally induced disease, which may be harmful to humans, must be considered. The ultimate responsibility for reducing or eliminating such risks lies with the PI.

Programs for the safe handling and ultimate disposition of potentially contaminated animals and animal wastes must protect the health and well-being of the employee, maintain the integrity of the experimental program, and minimize the hazard to non-program personnel or animals in adjacent areas. Such programs are based on an understanding of the hazard potential involved in working with animals. Procedures, equipment, and facilities must be selected to minimize or eliminate such risks. A carefully conceived animal care program and properly designed animal facility are necessary to reduce biohazard exposure in animal facilities. Definitive procedures that encompass all potential exposure possibilities are beyond the scope of this document.

PIs are responsible for providing specific information to their personnel concerning the biohazardous agent involved (carcinogen, radioactive isotope, etc.), its host range, the ability of experimentally infected animals to infect non-exposed animals or to excrete the agent in urine or feces, special caging or animal isolation requirements, the need to autoclave isolation cages and their content prior to processing, and the selection and use of appropriate PPE.

2. Animal Blood and Blood Products

This section describes how to work safely with non-human primate and animal (non-primate) blood, body fluids, tissues, and cell lines.

a. Non-Human Primate Blood, Body Fluids, Tissues, and Cell Lines

Investigators working with non-human primates or non-human primate blood, body fluids, tissues, and cell lines should be concerned about safe-handling because of the extreme severity of some of the agents that primates can harbor without showing any clinical disease. Some of the agents that can result in fatal infections in humans are Macacine herpesvirus 1, Marburg virus, and *Shigella* spp. A significant proportion of monkeys have latent shigellosis and about 65% of Macaca spp. have antibodies to Macacine herpesvirus 1.

In addition, in September 1992, the CDC reported that two laboratory workers seroconverted following occupational exposure to simian immunodeficiency virus (SIV), a lentivirus that causes acquired immunodeficiency syndrome (AIDS)-like illnesses in susceptible Macaca spp.

The same blood and body fluid precautions used for humans (Appendix A) must consistently observed with all specimens from non-human primates. All laboratory personnel must be familiar with these precautions prior to working with primate body fluids.

b. Animal (non-primate) Blood, Body Fluids and Tissues, and Cell Lines

Non-primates generally present a less immediate hazard potential than do primates. However, bats, dogs, cats, rabbits, rats, mice, etc. can carry microorganisms that are infectious to humans. In particular, animals acquired from unregulated sources must be considered a potential source of infection. For example, dogs and cats can carry rabies. Other infectious agents may be present without producing clinical illness in the animal. Generally, the same good laboratory practices used when working with primate source materials are followed when working with non-primate blood, body fluids, and tissue.

3. Animal Biosafety Levels and Vivarium Research Facilities

As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with biohazardous agents *in vivo* and *in vitro* are comparable. All facility requirements discussed for biosafety laboratories in Section 4.A.3 apply to research with animals as well. The animal room can present unique problems. The activities of the animals themselves can present special hazards not found in standard microbiological research laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. All additional animal facility SOPs must be followed.

The Animal Biosafety Levels 1-4 in *NIH Guidelines*: Appendix G and *BMBL* describe in detail the animal facilities and practices applicable to work with animals that have been infected with agents assigned to Biosafety Levels 1-4. These four biosafety combinations provide increasing levels of protection to research staff and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. There is no research requiring ABSL-4 containment at the UW.

Existing standards and regulations govern animal facilities, operational practices, and the quality of animal care. These standards and regulations are beyond the scope of this manual. Additional information on those aspects of animal facilities is available from the IACUC.

Animals that have received a biohazardous agent should be housed in separate animal rooms, preferably in limited access rooms on a separate ventilation system. Animal room doors, as well as individual cages, should be conspicuously labeled with information regarding the agent used, date of exposure, the biohazard symbol, and the names and telephone numbers of the PI and responsible technician.

4. Animal Work Practices and Engineering Controls

The following work practices and engineering controls apply in addition to the biosafety practices discussed in Section 4.B of this manual. *BMBL*: Section V. Table 3 provides a summary of recommended animal biosafety levels for activities in which experimentally or naturally infected vertebrate animals are used.

a. Gloves **-** Personnel who handle animals must wear gloves appropriate for the task. Hands must be washed after gloves are removed.

b. Additional PPE **-** Personnel handling animals that have received biohazardous agents must wear a face mask, gloves, and gown or other appropriate PPE.

c. Animal cages **-** Animals that are infected with a biohazardous agent are isolated within specific barriers such as filter-top cages, isolation racks, or ventilated racks. In all of these systems, the effectiveness of the barrier is determined by its design and the personnel using it. Thus, employee training is of paramount importance.

d. Transport of animals **-** Extreme care must be taken in transferring animals from biohazard animal rooms to laboratories or other facilities. Personnel should wear proper masks, gloves, gowns, caps, and footwear. The animal must be in a sealed container (or filter-top cage) and transport equipment must be sanitized or sterilized immediately after transport.

e. Necropsy - Postmortem dissection or necropsy is often performed on laboratory animals. Personnel conducting necropsies must wear appropriate PPE.

   Post-mortem examinations of small animals exposed to biohazards should be conducted in Class II BSCs when possible. If such equipment is not available, extreme care must be taken to guard against the creation of aerosols and the contamination of conventional necropsy facilities.

   The necropsy table should be stainless steel and have suitable flushing devices.

   Appropriate disinfectant should be used to completely and thoroughly disinfect all instruments and working surfaces that come into contact with animal tissues.

f. Perfusions **-** Perfusions of animals infected with biohazardous agents must be performed in a fume hood or a non-recirculating BSC.

5. Occupational Health Program

All employees assigned to animal facilities, or having significant contact with animals or potentially contaminated animal wastes, should have pre-employment and periodic medical examinations. This service is provided by Employee Health Services.

The Animal Use Medical Screening (AUMS) program is a component of the UW's animal use Occupational Health and Safety Program required by Federal authorities. For more information on the UW AUMS program refer to Section 5 of this manual.

6. Pest Control Program

The University provides a pest control program to control or eliminate crawling and flying insects, wild rodents, or similar pests. All pest associated breeding sites should be sealed or eliminated. Pesticides or traps are to be used as appropriate in conjunction with a strict program of sanitary maintenance. To prevent toxic effects and possible interference with experimental procedures, pesticides (including insecticide-impregnated plastics) must be administered by a licensed professional. Contact EH&S Public Health at 206-616-1623 or 206-543-7209 concerning pest control issues.

7. Animal Waste Handling Procedures

Animal waste collection and disposal should be scheduled on a regular and timely basis. When storage of animal waste is required, the area selected should be physically separate from other storage facilities and free of insects and rodents. Refrigerated storage facilities are recommended when waste must be held in excess of four to six hours.

a. Disposal of Animal Carcasses and Body Parts

Animal carcasses and animal body parts are a type of biological waste that requires special handling depending on whether it is radioactive, infectious, or non-hazardous. Procedures may also vary depending on location. Consult the location specific Biohazardous Waste Flow Charts for your location to determine how to dispose of animal carcasses and parts.

b. Disposal of Animal Blood and Blood Products

Animal blood and blood products and animal waste/bedding from animals infected with recDNA or other biohazardous agents are handled as biomedical waste that can be chemically decontaminated or autoclaved according to established guidelines prior to disposal. The Biohazardous Waste Flow Charts describe the process. In particular, blood, blood products, tissue, and tissue suspension, including blood contaminated items, must be decontaminated prior to disposal. Exempted are small amounts of non-primate blood, which can be flushed down sink drains without chemical treatment.

Additional information on waste disposal is found in Section 4.F.

E. DECONTAMINATION

1. Introduction

The primary target of decontamination is the microorganism that is under active investigation. Laboratory preparations of infectious agents usually have titers grossly in excess of those normally observed in nature. The decontamination of these high-titer materials presents certain problems.

Maintenance systems for bacteria or viruses are specifically selected to preserve the viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interference with the desired action of decontaminants may require the use of decontaminant concentrations and contact times in excess of those shown to be effective in the test tube.

Similarly, a major portion of decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these survivors. These problems are, however,

common to all potentially infectious agents and must always be considered in selecting decontaminants and procedures for their use.

2. Resistance

Microorganisms exhibit a range of resistance to chemical decontaminants. In terms of practical decontamination, most vegetative bacteria, fungi, and lipid-containing viruses are relatively susceptible to chemical decontamination. The non-lipid-containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Bacterial spores are the most resistant.

The relative resistance to the action of chemical decontaminants can be substantially altered by factors such as concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility, within the limits of sensitivity of the assay systems employed.

3. Ineffectiveness

Ineffectiveness of a decontaminant is due primarily to the failure to contact the microorganisms rather than failure of the decontaminant to act. If an item is placed in a liquid decontaminant, the item becomes covered with tiny bubbles. The area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. If there are spots of grease, rust, or dirt on the object, microorganisms under these protective coatings will also not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful.

4. Residual Action

Many chemical decontaminants have residual properties that may be considered a desirable feature in terms of aiding in the control of background contamination. However, consider residual properties carefully. Ethylene oxide can leave residues which cause skin irritation. In a concentrated form, phenol readily penetrates the skin and causes severe burns. Animal cell cultures, as well as viruses of interest, are also inhibited or inactivated by decontaminants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in liquid decontaminants prior to autoclaving and cleaning require careful selection of detergents for washing and must be thoroughly rinsed.

5. Exposure Time

Specific exposure times for the decontamination of soiled items by autoclaving, dry heat, or chemical decontaminants cannot be specifically stated. The volume of material treated, its contamination level, the soil load and type(s), moisture content, and other factors all play a role in the inactivation rate of microorganisms.

Inactivation of microorganisms by chemical decontaminants may be achieved in one or more of the following ways:

a. Coagulation and denaturation of protein

b. Lysis

c. Binding to enzymes, inactivation of an essential enzyme by binding, or destruction of enzyme substrate

d. Oxidation

Dozens of decontaminants are available under a wide variety of trade names. Table 2 provides information on commonly used laboratory decontaminants. A decontaminant selected on the basis of its effectiveness against microorganisms on any range of the resistance scale will be effective against microorganisms lower on the scale. Therefore, if decontaminants that effectively control spores are selected for routine laboratory decontamination, it can be assumed that any other microorganisms generated by laboratory operations, even in high concentrations, would also be inactivated.

Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic matter in the material being decontaminated. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times.

It should be emphasized that these data are only indicative of efficacy under artificial test conditions. The efficacy of any of the decontaminants should be conclusively determined by individual PIs. It is readily evident that each of the decontaminants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of decontaminants.

To assist in the selection of an appropriate decontaminant, consider the answers to the following questions:

- What is the target microorganism(s)?
- What decontaminants, in what form, are known to, or can be expected to, inactivate the target microorganism(s)?
- What degree of inactivation is required?
- Is the situation complicated by the presence of organic matter such as blood, agar, etc.?
- What types of surfaces are being targeted: solid or porous and/or airborne?
- What is the highest concentration of cells anticipated to be encountered?
- Can the decontaminant, either as an aqueous solution, a vapor, or a gas, reasonably be expected to contact the microorganisms and can effective duration of contact be maintained?
- What restrictions apply with respect to compatibility of materials?
- Do the anticipated procedures require immediate availability of an effective concentration of the decontaminant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?
- Will the toxicity of the decontaminant harm the researcher or other workers in the area?

Several terms are used when discussing decontamination. Sterilization refers to methods that destroy all forms of microbial life. Disinfection refers to methods that remove or destroy pathogens. It is important that the distinction between the two terms be understood. Sanitization refers to methods that reduce the level of microorganisms. Additionally, it is useful

to know that the ending "cide" (as in "bactericide") refers to killing, while the ending "stat" (as in "bacteriostat") refers to inhibiting growth.

Table 1: Summary of Practical Laboratory Decontaminants

Chemical Decontaminants	Concen-trations	Active Ingredient Concentration	Contact Time	Minutes required to inactivate bacterial spores	Minutes required to inactive lipid viruses	Agents Inactivated [3]	Vegetative Bacteria	Lipid Virus	Non-lipid Virus	Bacterial Spores	Slow Virus	Characteristics [4]	Effective Shelf life > 1 week	Corrosive	Flammable	Explosion Potential	Residue	Inactivate by organic matter	Microscope & camera lens compatible	Electronics compatible Skin irritant	Eye irritant	Respiratory irritant	Toxic	Potential Uses [5]	Work surfaces	Dirty Glassware	Liquids for Discard to Sewer	Portable equipment surface decon	Portable equipment penetrating decon	Stationary equipment surface decon	Stationary equipment penetrating decon	Lenses and electronic instruments	Large are decon	Air handling systems	Books and papers	Common Trade Names
Chlorine Compounds	5250 ppm		30	10			XX	XX	XX	XX	X		No	Y			Y	Y			Y	Y	Y		U	U	U	U		U					Bleach
Iodophor	75-750 ppm		30	10			XX	XX	XX	XX	X		Y	Y			Y	Y			Y		Y		U			U		U					Wescodyne, Biocide
Formaldehyde	1-8%		30	10			XX	XX	XX	XX	-		Y				Y				Y	Y	Y					U		U					Formalin
Ethyl Alcohol	85%		N.E.	10			XX	X	X	-	-		Y		Y						Y		Y					U		U					
Isopropyl Alcohol	70%		N.E.	10			XX	X	X	-	-		Y		Y						Y		Y					U		U					
Phenolic compounds	2%		N.E.	10			XX	XX	X	-	-		Y	Y			Y	FT			Y		Y		U			U		U					Staphene, Amphyl
Quaternary ammonium compounds	2%		N.E.	10			X	XX	-	-	-		Y				Y	Y			Y		Y					U		U					Megasol
Hydrogen Peroxide	3-6%		30	10			XX	XX	FT	X	FT		Y								Y		Y					U		U		U	FT	FT	Liquid or Vapor
Glutaraldeyde	0.02		30	10			XX	XX	XX	XX	-		Y				Y	FT	Y		Y	Y	Y					U		U		U			Cidex, Procide, Metricide
Peracetic Acid	0.02		30	10			XX	XX	XX	XX	FT		Y								Y	Y	Y					U		U					
Ethylene Oxide[1]	45 gram/liter		60	60			XX	XX	XX	XX	FT		N/A		Y	Y	Y		Y	Y	Y	Y	Y						U			U			
Paraformaldehyde[2]	3 gram/ cu.ft		60	60			XX	XX	XX	XX	-		N/A		Y	Y			Y	Y	Y	Y	Y							U	U	U			

N.E.=NOT EFFECTIVE

FT=REQUIRES FURTHER TESTING

N/A=NOT APPLICABLE

1. REQUIRES TEMPERATURES OF 37^{0}F AND 30% RELATIVE HUMIDITY
2. REQUIRES TEMPERATURES OF 23^{0}C AND > 60% RELATIVE HUMIDITY
3. XX=GOOD
   X=FAIR TO GOOD DEPENDING
4. Y=HAS INDICATED CHARACTERISTICS.
   SEE MSDS
5. U=POTENTIAL USE

6. Sterilization

a. Wet Heat (Autoclave or Steam Sterilizer)

The use of an autoclave or steam sterilizer is the preferred method for treating biohazardous waste as well as decontaminating labware. See Section 4.F.5 for additional information on autoclaving.

b. Dry Heat

The use of dry heat for the decontamination of biohazardous materials and contaminated items is less efficient than autoclaving and requires a longer exposure time with higher temperature. It may be possible to decontaminate materials or soiled items by exposing them to 160°C (320°F) for four hours. This is suitable for destruction of viable agents on impermeable non-organic material such as glass but is not reliable in even shallow layers of organic material that can act as insulation. If items are heat sensitive, a temperature of 120°C (248°F) can be used and the exposure time necessary for decontamination is usually greater than 24 hours.

The use of biological indicators with dry heat is necessary to determine the most effective temperature and/or exposure time for decontamination of materials or equipment. The proper organism to use for challenging a gas or dry heat sterilization process is *Bacillus subtilis* var. The test protocol used is found above (under wet heat) except that the spore strips are incubated at 370°C according to manufacturer's instructions.

c. Chemical Sterilant

In the laboratory, chemical decontamination is necessary because the use of pressurized steam, the most rapid and reliable method of sterilization, is not normally feasible for decontaminating large spaces, surfaces, and stationary equipment. Moreover, high temperatures and moisture will damage delicate instruments.

1) Ethylene Oxide

   Ethylene oxide is not a practical decontaminant in most laboratory settings due to the potential toxic exposure to the worker. Ethylene oxide is used in hospital sterilizers for sterilizing heat sensitive equipment. The sterilizers are provided with dedicated ventilation canopies and monitoring equipment.

2) Paraformaldehyde and Formaldehyde Gas

   Formaldehyde gas can be liberated by heating paraformaldehyde to depolymerize it. This vapor is an effective space decontaminant for decontaminating biological safety cabinets, rooms or buildings, but in the vapor state with water, it tends to polymerize on surfaces and form paraformaldehyde, which remains persistent.

   In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms fewer polymerized residues on surfaces and less time is required to clear treated areas of fumes than formaldehyde released in the vapor state.

   The pungent, irritating odors and its classification as a potential cancer hazard limit the use of formaldehyde in the laboratory. Refer to the UW Laboratory Safety Manual for information on chemical hazards.

3) Glutaraldehyde

   In the past, 1%-2% solutions of glutaraldehyde have been used as cold sterilants on instruments that could not be heated. These solutions are being replaced by peratic acid solutions due to concerns about the toxicity of the glutaraldehydes.

7. Disinfection

The following chemical decontaminants are recommended when attempting to chemically disinfect materials. Disinfection is defined as destroying certain pathogens.

a. Halogens

The halogens (chlorine, iodine, bromine, and fluorine) will rapidly kill bacterial spores, viruses, rickettsiae, and fungi. These decontaminants are effective over a wide range of temperatures. In fact, chlorine has been shown to be effective at -400^{0}F. (On the other hand, phenols and formaldehyde have high temperature coefficients.) The halogens have several undesirable features. They readily combine with protein, so that an excess of the halogen must be used if proteins are present. The halogens are relatively unstable so that fresh solutions must be prepared. The frequency of preparation is discussed below. Finally, the halogens corrode metals. A number of manufacturers of decontaminants have treated the halogens to remove some of the undesirable features. For example, sodium hypochlorite reacts with paratoluenesulfonamide to form Chloramine T, and iodine reacts with certain surface-active agents to form the popular iodophors. These "tamed" halogens are stable, non-toxic, odorless, and relatively noncorrosive to metals. However, the halogens are highly reactive elements and, because they are reactive, they are good germicides. When a halogen acts as a decontaminant, free halogen is the effective agent. Raising the pH or combining the halogen with other compounds to decrease the corrosive effect also decreases the decontaminating effect of a halogen.

1) Chlorine

This halogen is a universal decontaminant that is active against all microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent and corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared unless the free available chlorine in the solution is checked. Currently, there are no practical means of verifying the level of free available chlorine at the level used in the laboratory. Sodium hypochlorite is usually used as a base for chlorine decontaminants and is more stable than other forms of chlorine. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25% available chlorine or 52,500 ppm. If they are diluted one to ten, the solution will contain 5,250 ppm of available chlorine. Diluting household bleach (sodium hypochlorite) with water produces a solution with lower pH and more free available chlorine. This solution is appropriate for sanitizing items or surfaces with high levels of organic matter. A one to 100 dilution with 525 ppm of available chlorine is appropriate for sanitizing items or surfaces with low levels of organic matter.

Bleach must never be mixed with ammonia or hydrochloric acid containing cleaners because toxic fumes are created.

2) Iodine

The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants used in the laboratory is the iodophors; Wescodyne is perhaps the most popular. The dilution range of Wescodyne recommended by the manufacturer is one ounce in five gallons of water (which yields 25 ppm of available iodine) to three ounces in five gallons (which yields 75 ppm. At 75 ppm, the concentration of free iodine is .0075%. This small amount can be rapidly taken up by any extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm

available iodine. However, difficulties may be experienced if any appreciable amount of protein is present. For bacterial spores, a dilution of one to 40 (which yields 750 ppm) is recommended by the manufacturer. There are test strips available commercially to verify the strength of available iodine in the range of 25-75 ppm. These strips can be used in verifying the stability of prepared solutions.

b. Formalin

Formaldehyde for use as a decontaminant is usually marketed as a 37% solution referred to as formalin. Formaldehyde in a concentration of 5% active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures.

Formalin has many of the same hazards mentioned for paraformaldehyde.

c. Hydrogen Peroxide and Peracetic Acid

Both are fast acting and effective. They are useful in the decontamination of medical instruments when used in specially designed washing units.

Additional information on decontaminants can be found in "Disinfection, Sterilization and Preservation" by S.S. Block (4th edition).

8. Sanitization

Sanitization refers to reducing the level of microorganisms present.

a. Alcohol

Ethyl or isopropyl alcohol in a concentration of 70-80% by weight is often used. Alcohols denature proteins and are somewhat slow in their action. They are effective decontaminants against many vegetative bacteria and lipid-containing viruses; however, they are not effective for viruses such as hepatitis B, spore forming bacteria, or the vegetative form of some gram negative organisms.

One of the disadvantages of the use of alcohols is their flammability. They should not be used in operations which require the use of an open flame.

b. Quaternary Ammonium Compounds or Quats

These compounds are not effective against gram negative organisms. Quats are cationic detergents, strongly surface-active, and effective against viruses containing lipids. Quats will attach to protein so that dilute solutions of quats will quickly lose effectiveness in the presence of proteins. Quats tend to clump microorganisms and are neutralized by anionic detergents such as soap. Quats have the advantages of being non-toxic, odorless, nonstaining, non-corrosive to metals, stable, and inexpensive.

c. Phenolic Compounds

Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against some viruses, rickettsiae, fungi, and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.

d. UV Light

UV (ultra-violet) light has limited effectiveness as an air decontaminant. It should never be relied upon to provide sole decontamination of surfaces. Additional surface decontamination with a chemical decontaminant is always warranted. UV lamps lose their effectiveness over time and when covered by a film of dust, etc. The lamp in a BSC should only be used when no one is in the room. UV light damages eyes, skin, and certain plastic materials.

9. General Procedures

a. Biohazardous liquid and solid wastes, as well as all items such as labware, equipment, or apparatuses contaminated with biohazards, must be decontaminated before being washed, sorted, or discarded.

Each individual working with biohazardous material or contaminated items is responsible for its decontamination.

b. Whenever possible, contaminated items or biohazardous liquid or solid waste should be decontaminated by autoclaving.

c. All floors, laboratory benches, and other surfaces or areas where biohazardous materials are handled should be chemically decontaminated as often as deemed necessary by the PI/lab manager. The choice of the chemical decontaminant used is at the discretion of the PI/lab manager.

d. Upon completion of operations involving plating, pipetting, centrifugation, and similar procedures with biohazardous materials, the surrounding area should be chemically decontaminated.

e. Floors should be wet mopped. Dry sweeping and dusting leads to the formation of secondary aerosols.

f. Stock solutions of suitable chemical decontaminants should be maintained on each laboratory bench.

10. Selecting Chemical Decontaminants for Research on recDNA Molecules

Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Oxidizing agents such as bleach will destroy the nucleic acid. However, the chosen decontaminant's ability to destroy the nucleic acid should be confirmed in the laboratory.

F. BIOHAZARDOUS WASTE

1. Responsibility

PIs are responsible for developing protocols for identifying, segregating, and decontaminating biohazardous waste, including all recDNA waste, prior to disposal.

Biohazardous waste disposal at the UW is dependent upon the location of waste generation. Refer to the Biohazardous Waste Flow Charts on the EH&S website for your location.

2. Identifying Biohazardous Waste

The following materials are defined as biohazardous (biomedical) waste:

a. Sharps waste: The sharps waste stream is regulated by state law; they must not be disposed of in the regular waste stream. The term "sharps" is a regulatory waste classification associated with those instruments used to puncture, cut, or scrape body parts and that, in a waste container, can cause punctures or cuts to solid waste handlers or to the public. This means that all sharps waste should be placed in appropriate sharps containers and decontaminated prior to disposal.

Sharps include the following:

1) Needles, including syringes with needles and IV tubing with needles attached

2) Syringes without needles, when removed from their original sterile containers

3) Lancets

4) Scalpel blades

5) Other sharps items not defined above only if contaminated with biohazardous material including recDNA (e.g. broken glass; razor blades; fragile glass tubes, vials, or ampoules including glass Pasteur pipettes; glass slides and cover slips)

b. Human and non-human primate blood, blood products, body fluids, tissues, and cells: includes human and non-human primate blood, blood components, and materials containing free-flowing blood and blood products. Both human and non-human primate cell lines, regardless of origin, are also defined as biohazardous waste.

c. Cultures and stocks of etiologic agents and associated biologicals: include but are not limited to specimen cultures, discarded live and attenuated vaccines, cultures and stocks of etiologic agents, and wastes from the production of biologicals and serums.

d. RecDNA: includes but is not limited to waste products from laboratory research procedures involving recDNA in plasmids, viral vectors, *E. coli*, yeast, cell cultures, as well as naked DNA from polymerase chain reaction (PCR) and sequencing reactions. This also includes tissue and cells harvested from animals containing recDNA (e.g., transgenic animals).

e. Laboratory waste that has come in contact with a biohazard as listed in a, b, c, or d. above: includes but is not limited to culture dishes, blood specimen tubes, devices used to transfer, inoculate, and mix cultures, and other materials that have come in contact with biohazards (including disposable PPE and clothing).

f. Animal waste, animal carcasses, and body parts exposed to pathogens or recDNA: includes animal bedding and other waste from such animals and all non-human primate tissue and carcasses.

g. Human pathological waste: includes human source biopsy materials, tissues, and anatomical parts. This does not include teeth, human corpses, remains, and anatomical parts that are intended for interment or cremation.

h. Plant waste: includes all transgenic plants, seeds, spores, plant debris, and soil materials, plus any plants exposed to plant pathogens.

3. Collection and Handling of Biohazardous Waste

a. Sharps Container Waste

Sharps must be contained in leak proof, rigid, puncture-resistant, durable plastic containers. These containers are red in color and equipped with a tight-fitting lid for use during handling and transport. (Refer to Section 4.F.2 for additional information on sharps).

Treatment and disposal of sharps waste generated at the Seattle campus and other UW offsite research locations is dependent upon the location of the waste generation. Refer to the location-specific Biohazardous Waste Flow Charts for details.

Sharps containers must be autoclaved when 2/3 filled. When autoclaving on-site, place a strip of autoclave tape running from the lid and down across the biohazard symbol on the container to secure the lid prior to autoclaving. Do not cover the vent holes on the lid during the autoclave cycle. Label the container with the room number and the PI's name. Sterilized sharps containers must be segregated from regular waste containers at all

times. Follow your location-specific procedures for collection of sterilized sharps containers.

For additional information on segregation and handling, refer to the EH&S page on sharps and laboratory glass and plastic waste.

b. Liquid Biohazardous Waste

Liquid biohazardous (including recDNA) waste containing free flowing liquids is contained in leak proof, rigid, durable containers labeled with the biohazard symbol and the word "biohazard." These containers are closed and placed in leak proof containers for handling or transport.

Liquid wastes must not be disposed of as solid waste. Liquid biohazardous waste (e.g., liquid recDNA waste, liquid pathogenic waste, small amounts of human and non-human primate blood, blood products, and other free-flowing body fluids) must be treated prior to disposal in the sewer system EITHER by:

- Bleaching **-** Add freshly prepared chlorine bleach to a final concentration of 10% bleach. The solution must sit for at least 30 minutes prior to disposal in the sewer;

  Or

- Autoclaving **-** Remove or loosen caps before loading into the autoclave. Follow autoclaving instructions as outlined in # 5 below. After the autoclaved liquid has cooled, dispose of the fluid in the sewer.

Disposal of human and non-human primate blood, blood products, and other free-flowing body fluids in 10 liter or greater volumes: Prior to disposing of human and non-human primate blood, blood products, and other free-flowing body fluids in 10 liter or greater volumes, contact EH&S ROS at 206-221-7770.

Animal blood other than primate blood**:** Small quantities may be flushed into the sewer system without treatment. Due to coagulation when handling large quantities, flushing is impractical. Contact EH&S ROS at 206-221-7770 for additional information on the disposal of large volumes of blood. If the blood and/or body fluid is potentially infected with a pathogen, handle according to the guidelines for human blood.

c. Solid Biohazardous Waste

Solid recDNA or other biohazardous waste (e.g., contaminated gloves, culture dishes) is collected in the laboratory in plastic, autoclavable, biohazard waste bags which must be contained inside a rigid container. The outer container must be labeled or clearly display the bag's biohazard symbol. A polypropylene bin can be used to hold biohazardous waste bags and can be placed in the autoclave with the waste bags. The recommended closure device is a loosely tied rubber band. Refer to the EH&S page on biohazardous waste for additional information on segregation and packaging of biohazardous waste.

The biohazardous waste bags must be decontaminated by autoclave prior to disposal within 14 days from the first generation of the waste. Bagged waste disposal, like all biological waste at the UW, is dependent upon the location of waste generation. Refer to the location-specific Biohazardous Waste Flow Charts for details.

Transport the biohazardous waste bags to autoclave in a rigid, leak-proof secondary container. Autoclave tape must be used on biohazard bags to notify Custodial Services that the waste has been treated. After autoclaving, bagged waste may be placed alongside the regular waste container for the laboratory.

Human pathological waste: Incinerate. This material must never be disposed of in the general waste stream. Arrangements for disposal are made through the Autopsy Coordinator at the UW Medical Center.

Non-human primate pathological waste**:** Incinerate. Arrangements for disposal are made through the WaNPRC, 206-543-8686.

Animal carcasses, animal parts, bedding, and waste**:** The disposal for all these materials is coordinated through the Department of Comparative Medicine. That department must be consulted for current guidelines for packaging, handling, and returning these materials to their facility for disposal. You may also refer to the location-specific Biohazardous Waste Flow Charts for disposal of these materials at your location.

Radioactive/biohazardous animals**:** Contact EH&S Radiation Safety at 206-543-0463 for additional information.

Mixed biological waste and chemical waste**:** Should be avoided. Contact EH&S ROS at 206-221-7770 prior to generating the waste.

Non-infectious/non-recombinant/non-radioactive animals are disposed by the Department of Comparative Medicine. You may also refer to the location-specific Biohazardous Waste Flow Charts for disposal of these materials at your location.

Radioactive/non-infectious animals: Dead animals treated with radioactive materials are disposed of in accordance with procedures listed in the UW Radiation Safety Manual.

Other biohazardous (biomedical) waste**:** Contact EH&S ROS at 206-221-7770 for advice and assistance.

4. Transporting Biohazardous (including recDNA) Waste

This section outlines the proper procedures for transporting biohazardous waste within buildings and between buildings. Biohazardous waste must be packaged so that PPE is not needed during transport. If PPE is necessary, then the waste is not properly packaged.

a. Within Building

1) Sharps Containers

Sharps containers with contaminated sharps, transported within the same building, must be securely closed and the outer surface decontaminated prior to transport.

- Attach a piece of autoclave tape over the lid and sides being careful not to cover air vents. This will help secure the lid if the plastic expands and contracts during steam sterilization. Label the container with name of the PI and the room number.
- If leaking is possible, place inside a secondary container. The secondary container must be closable, puncture resistant, and constructed to contain all contents and prevent leakage. This container should either be red in color or labeled with the biohazard symbol.

2) Other Biohazardous Waste

Other biohazard waste that does not pose a threat of skin puncture shall be placed in plastic biohazard bags. Bagged biohazardous waste transported within the same building must be closed, surface decontaminated, and placed inside a secondary containment prior to transport.

- Tie or tape the biohazard bag closed (loosely to allow steam penetration during autoclaving).
- Place the bagged waste inside a rigid, leak-proof secondary container (e.g., autoclave tub, plastic container). Biohazardous waste cannot be transported in biohazard bags alone.

- If the secondary container is closed, it must be identifiable as biohazardous either by being red in color or labeled with the biohazard symbol.

3) Animal Carcasses

Contact the Department of Comparative Medicine prior to transporting animal carcasses. They will provide instruction in packaging and transportation.

b. Between Buildings

Sharps containers with contaminated sharps, transported between buildings, have the same requirements as within the same building (see above). The exception is if transport is by motor vehicle, which must be a UW owned and operated vehicle (e.g., Fleet Services, UCAR).

Bagged biohazardous waste transported between buildings has the same requirements as within the same building (see above), with the exception that the secondary container must have a secured lid.

1) Biohazard bags must be closed, surface decontaminated, and placed inside a leak proof secondary container with a secured lid prior to transport.

2) The secondary container must be identifiable as biohazardous either by being red in color or labeled with the biohazard symbol.

3) If using a motor vehicle for transport between buildings, the vehicle must be a UW owned and operated vehicle (e.g., Fleet Services, UCAR).

5. Autoclave Quality Control

A properly operating autoclave renders biohazardous waste sterile so that it can be disposed of safely via municipal waste. Any University of Washington laboratory or facility that uses an autoclave to decontaminate biohazardous waste is required to follow the General Autoclave Safety Guidelines, the Autoclaving Biohazardous Waste Guidelines, and implement a site-specific procedure for autoclaving biohazardous waste. Refer to the EH&S Autoclaves webpage.

The Seattle/King County Infectious Waste regulations require all operators of autoclaves to be trained. The training procedure must be written, and all users must have access to the written procedures. All users must receive this training prior to actually using the autoclave. It is the responsibility of the principal investigator or laboratory/facility manager, and/or department to ensure compliance with all autoclave safety guidelines and the UW Biohazardous Waste Management Plan. Autoclave records must be maintained for six years.

a. Autoclave Operation:

Consult the manufacturer's manual for your autoclave to select or program a cycle. For sterilization of biohazardous waste, the cycle must include a minimum temperature of 121°C or 250°F for 30 minutes or longer, depending on size and compaction of the load. The full cycle time will take 60-90 minutes. Greater time and/or temperature may be necessary to sterilize certain loads.

b. Autoclave Monitoring:

To ensure adequate waste sterilization, monitor each autoclave as follows:

1) Temperature – Each cycle, ensure autoclave has a recording and/or indicating thermometer or other method to verify temperature. Check and record that sterilization temperature (121°C) was achieved and sustained for at least 30 minutes. Calibrate the thermometer annually.

2) Heat-sensitive tape (autoclave tape) – Each cycle, use heat-sensitive tape to visually indicate steam sterilization. Tape only indicates that proper temperature was reached; it does not indicate if heat was sustained for sufficient time.

3) Chemical integrator – Each cycle, place an approved integrator in the center of the load to confirm attainment of adequate sterilization. Note: Thermalog-S and Steriscan are the only integrators approved for us by the Seattle-King County Health Department.

4) Biological indicator – At least monthly, use the biological indicator Bacillus stearothermophilus at the center of a load to confirm the attainment of adequate sterilization conditions. Instructions are included on the Quality Control Checklist.

5) Structural inspection – If autoclave is over five cubic feet in volume, contact Facilities Services Maintenance & Alterations for an autoclave structural inspection with a qualified inspector (required per WAC 296-104-100). Post sticker/sign indicating maximum permissible pressure and date of confirmation.

6. Refusal to Collect Waste

Custodial Services personnel have been instructed to refrain from removing any animal carcasses, parts, or other questionable wastes and to report discrepancies to their supervisors. The reports are referred to EH&S ROS, 206-221-7770, for resolution.

G. CONTROL OF recDNA EXPERIMENTATION

1. Responsibility

Procedures for obtaining approval for research involving the use of recDNA techniques at the UW can be found in Section 2.

When working with recDNA materials, effective biological safety programs involve selecting the appropriate biological containment method, including the physical containment facilities.

It is the responsibility of the PI to assess the potential risk associated with the experiment and determine an appropriate host-vector system (biological containment) and physical containment to be used for the proposed experiment. The IBC will review this assessment of risk.

2. Physical Containment

Standard microbiological practices are covered in Section 4.B of this manual.

Special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated risk are covered in Section 4.C.

3. Biological Containment

Experiments involving recDNA molecules, by their very nature, lend themselves to biological containment. Natural barriers exist which limit either the infectivity of a vector or vehicle (plasmid, bacteriophage, or virus) to specific hosts or its dissemination and survival in the environment.

The vectors that provide the means for replication of the recDNA and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recDNA molecules outside the laboratory.

In considering biological containment, the vector (plasmid or virus) for the recDNA and the host (bacteria, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together.

Discussion of the various levels of biological containment is beyond the scope of this manual. Additional information can be found in *NIH Guidelines*. Copies are also available from EH&S ROS at 206-221-7770.